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# INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : C12N 15/86, 5/10, 7/04, 15/49, C07K 14/16

A1

(11) International Publication Number: WO 00/15819

(43) International Publication Date: 23 March 2000 (23.03.00)

(21) International Application Number: PCT/US99/20675

(22) International Filing Date: 10 September 1999 (10.09.99)

(30) Priority Data:
60/100,022 11 September 1998 (11.09.98) US
60/100,063 12 September 1998 (12.09.98) US

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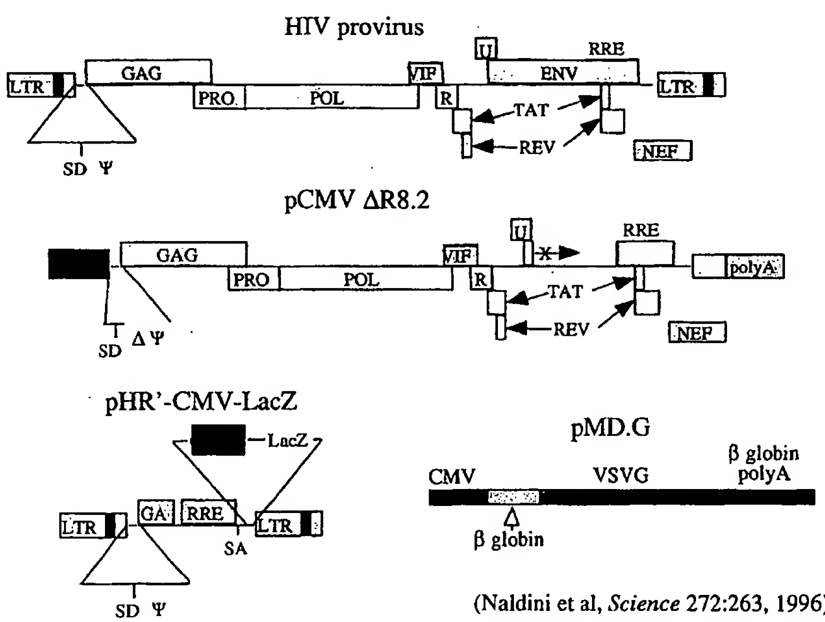
(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published

*With international search report.*
*Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.*

(54) Title: PACKAGING CELL LINES FOR HIV-DERIVED RETROVIRAL VECTOR PARTICLES




(Naldini et al, *Science* 272:263, 1996)

(57) Abstract

Novel packaging cell lines useful for generating viral accessory protein independent HIV-derived retroviral vector particles, methods of constructing such packaging cell lines and methods of using the viral accessory protein independent HIV-derived retroviral vector particles are disclosed.

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# PACKAGING CELL LINES FOR HIV-DERIVED RETROVIRAL VECTOR PARTICLES

## BACKGROUND OF THE INVENTION

Retroviral vectors based on lentiviruses, such as human immunodeficiency viruses (HIV), can infect nondividing cells, and integration of proviral DNA occurs without the need for cell division. These properties make lentiviruses attractive for gene transfer into nondividing cells, such as hepatocytes, myofibers, hematopoietic stem cells, and neurons.

However, the use of lentivirus vectors, particularly HIV vectors, particularly for gene therapy, is hampered by concern over their safety. Thus, a need for the development of lentivirus vectors, particularly HIV vectors, with improved safety, particularly for gene therapy, exists.

## SUMMARY OF THE INVENTION

The present invention relates to novel packaging cell lines useful for generating viral accessory protein independent lentivirus-derived, particularly HIV-derived, retroviral vector particles, to construction of such cell lines and to methods of using the accessory protein independent lentivirus-derived retroviral vector particles to introduce DNA of interest into cells (e.g, eukaryotic cells such as animal (particularly mammalian), plant or yeast cells or prokaryotic cells such as bacterial cells). In a preferred embodiment, the packaging cell lines of the present invention are stable packaging cell lines.

In one embodiment of the invention, packaging cell lines for producing a viral accessory protein independent lentivirus-derived retroviral vector particles comprise (a) a cell (e.g., mammalian cell); and (b) a retroviral nucleotide sequence in the cell which comprises a coding sequence for lentivirus *gagpol*, wherein said coding sequence has

been codon optimized by mutagenisis to improve expression of the lentivirus gagpol proteins.

In second embodiment of the invention, packaging cell lines for producing a viral accessory protein independent lentivirus-derived retroviral vector particles comprise (a) a cell (e.g., mammalian cell); (b) a first retroviral nucleotide sequence in the cell which comprises a coding sequence for lentivirus *gagpol*, wherein said coding sequence has been codon optimized by mutagenisis to improve expression of the lentivirus gagpol proteins; and (c) a second retroviral nucleotide sequence in the cell which comprises the coding sequence for a heterologous envelope protein.

In a third embodiment of the invention, packaging cell lines for producing a viral accessory protein independent lentivirus-derived retroviral vector particles comprise (a) a cell (e.g., mammalian cell); (b) a first retroviral nucleotide sequence in the cell which comprises a coding sequence for lentivirus *gagpol*, wherein said coding sequence has been codon optimized by mutagenisis to improve expression of the lentivirus gagpol proteins; (c) a second retroviral nucleotide sequence in the cell which comprises the coding sequence for a heterologous envelope protein; and (d) a third retroviral nucleotide sequence which comprises a DNA sequence of interest and lentivirus cis-acting sequences required for packaging, reverse transcription and integration.

In a fourth embodiment of the invention, packaging cell lines for producing a viral accessory protein independent lentivirus-derived retroviral vector particles comprise (a) a cell (e.g., mammalian cell); (b) a retroviral nucleotide sequence in the cell which comprises a coding sequence for lentivirus *gagpol*, wherein said coding sequence has been codon optimized by mutagenisis to improve expression of the lentivirus gagpol proteins; and (c) a retroviral nucleotide sequence which comprises a DNA sequence of interest and lentivirus cis-acting sequences required for packaging, reverse transcription and integration.

In a fifth embodiment of the invention, packaging cell lines for producing a viral accessory protein independent HIV-derived retroviral vector particles comprise (a) a cell

(e.g., mammalian cell); and (b) a retroviral nucleotide sequence in the cell which comprises a coding sequence for HIV *gagpol*, wherein said coding sequence has been codon optimized by mutagenisis to improve expression of the HIV gagpol proteins.

In sixth embodiment of the invention, packaging cell lines for producing a viral accessory protein independent HIV-derived retroviral vector particles comprise (a) a cell (e.g., mammalian cell); (b) a first retroviral nucleotide sequence in the cell which comprises a coding sequence for HIV *gagpol*, wherein said coding sequence has been codon optimized by mutagenisis to improve expression of the HIV gagpol proteins; and (c) a second retroviral nucleotide sequence in the cell which comprises the coding sequence for a heterologous envelope protein.

In a seventh embodiment of the invention, packaging cell lines for producing a viral accessory protein independent HIV-derived retroviral vector particles comprise (a) a cell (e.g., mammalian cell); (b) a first retroviral nucleotide sequence in the cell which comprises a coding sequence for HIV *gagpol*, wherein said coding sequence has been codon optimized by mutagenisis to improve expression of the HIV gagpol proteins; (c) a second retroviral nucleotide sequence in the cell which comprises the coding sequence for a heterologous envelope protein; and (d) a third retroviral nucleotide sequence which comprises a DNA sequence of interest and HIV cis-acting sequences required for packaging, reverse transcription and integration.

In a eighth embodiment of the invention, packaging cell lines for producing a viral accessory protein independent HIV-derived retroviral vector particles comprise (a) a cell (e.g., mammalian cell); (b) a retroviral nucleotide sequence in the cell which comprises a coding sequence for HIV *gagpol*, wherein said coding sequence has been codon optimized by mutagenisis to improve expression of the HIV gagpol proteins; and (c) a retroviral nucleotide sequence which comprises a DNA sequence of interest and HIV cis-acting sequences required for packaging, reverse transcription and integration.

Alternatively, each of the packaging cell lines described herein can be produced using (1) a retroviral nucleotide sequence which comprises a codon optimized gag

coding sequence and (2) a retroviral nucleotide sequence which comprises a codon optimized pol coding sequence, in place of the retroviral nucleotide sequence which comprises a codon optimized gagpol coding sequence.

In a particular embodiment, the heterologous envelope protein is the G glycoprotein of vesicular stomatitis virus (VSV G). In another embodiment, the heterologous envelope protein is the amphotropic envelope of the Moloney leukemia virus (MLV).

Cell lines for producing a viral accessory protein independent lentivirus-derived retroviral vector particles are produced by transfecting host cells (e.g., mammalian host cells) with a plasmid comprising a DNA sequence which encodes lentivirus *gagpol* proteins, wherein said DNA sequence has been codon optimized by mutagenisis to improve expression of the lentivirus gagpol proteins. Depending upon the particular cell line being produced, the host cells are also co-transfected with a plasmid comprising a DNA sequence which encodes a heterologous envelope protein, or a plasmid comprising a DNA sequence of interest and lentivirus cis-acting sequences required for packaging, reverse transcription and integration, or both of these plasmids. Alternatively, host cells are transfected with a plasmid comprising a codon optimized DNA sequence encoding a lentivirus gag protein and a plasmid comprising a codon optimized DNA sequence encoding a lentivirus pol protein, in place of the plasmid comprising a codon optimized DNA sequence encoding both lentivirus gagpol proteins.

Cell lines for producing a viral accessory protein independent HIV-derived retroviral vector particles are produced by co-transfecting host cells (e.g., mammalian host cells) with a plasmid comprising a DNA sequence which encodes HIV *gagpol* proteins, wherein said DNA sequence has been codon optimized by mutagenisis to improve expression of the HIV gagpol proteins. Depending upon the particular cell line being produced, the host cells are also co-transfected with a plasmid comprising a DNA sequence which encodes a heterologous envelope protein, or a plasmid comprising a DNA sequence of interest and HIV cis-acting sequences required for packaging, reverse

transcription and integration, or both of these plasmids. Alternatively, host cells are transfected with a plasmid comprising a codon optimized DNA sequence encoding a HIV gag protein and a plasmid comprising a codon optimized DNA sequence encoding a HIV pol protein, in place of the plasmid comprising a codon optimized DNA sequence encoding both HIV gagpol proteins.

The present invention also relates to methods of producing viral accessory protein independent lentivirus-derived retroviral vector particles, comprising co-transfecting host cells (e.g., mammalian host cells) with (a) a first plasmid comprising a DNA sequence which encodes lentivirus *gagpol* proteins, wherein said DNA sequence has been codon optimized by mutagenisis to improve expression of the lentivirus gagpol proteins; (b) a second plasmid comprising a DNA sequence which encodes a heterologous envelope protein; and (c) a third plasmid comprising a DNA sequence of interest and lentivirus cis-acting sequences required for packaging, reverse transcription and integration. Alternatively, host cells are transfected with a plasmid comprising a codon optimized DNA sequence encoding a lentivirus gag protein and a plasmid comprising a codon optimized DNA sequence encoding a lentivirus pol protein, in place of the first plasmid comprising a codon optimized DNA sequence encoding both lentivirus gagpol proteins.

In a particular embodiment, the invention relates to methods of producing viral accessory protein independent HIV-derived retroviral vector particles, comprising co-transfecting host cells (e.g., mammalian host cells) with (a) a first plasmid comprising a DNA sequence which encodes HIV *gagpol* proteins, wherein said DNA sequence has been codon optimized by mutagenisis to improve expression of the HIV gagpol proteins; (b) a second plasmid comprising a DNA sequence which encodes a heterologous envelope protein; and (c) a third plasmid comprising a DNA sequence of interest and HIV cis-acting sequences required for packaging, reverse transcription and integration. Alternatively, host cells are transfected with a plasmid comprising a codon optimized DNA sequence encoding a HIV gag protein and a plasmid comprising a

codon optimized DNA sequence encoding a HIV pol protein, in place of the first plasmid comprising a codon optimized DNA sequence encoding both HIV gagpol proteins.

The present invention also relates to viral accessory protein-independent retroviral particles produced by or obtainable by (obtained by) the methods described herein.

The present invention further relates to isolated DNA encoding a codon optimized lentivirus *gagpol*, isolated DNA encoding the *gag* coding region of a codon optimized lentivirus *gagpol*, and isolated DNA encoding the *pol* coding region of a codon optimized lentivirus *gagpol*. In a particular embodiment, the present invention relates to isolated DNA encoding a codon optimized HIV *gagpol*, isolated DNA encoding the *gag* coding region of a codon optimized HIV *gagpol*, and isolated DNA encoding the *pol* coding region of a codon optimized HIV *gagpol*.

The packaging cell lines and viral particles of the present invention can be used for gene therapy or gene replacement with improved safety. The packaging cell lines and viral particles of the present invention can also be used in development and production of vaccines, and in production of biochemical reagents. Gene therapy vectors produced with the cell lines of the present invention are expected to be valuable medical therapeutics.

## BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic diagram of an expression cassette containing the codon optimized *gagpol* genes. The DNA was constructed in multiple segments, which are indicated at the top as 1/3, 2/3, 3/3 (A, B, C and D) and HIN. Restriction sites used to assemble the cloned segments are indicated above the kilobasepair (Kb) ruler. Below the ruler are multiple features showing the location of the human cytomegalovirus (CMV) promoter, human betaglobin sequences (Bglobin), mRNA sequences (thinner line represents intronic sequence), the *gag* and *pol* open reading frames, the individual

proteolytic fragment coding sequences (p17_MA, p24_CA, p7, p6, PR, p51_RT, RNaseH and integrase (IN)) and each synthetic oligonucleotide used in the assembly process (multiple adjacent open arrows).

Figure 2 is a table which depicts codon usage frequencies in genes which are highly expressed and in the codon optimized gagpol open reading frame of the HIV packaging construct described herein.

Figure 3 is a schematic representation of the HIV provirus and a three-plasmid expression system used for generating a pseudotyped HIV-based vector by transient transfection as described in Naldini *et al.*, *Science, 272:*263-267 (1996).

Figure 4 is a list of some characteristics relating to the HIV Rev protein.

Figure 5 is a list of some points relating to codon optimization of HIV *gagpol*.

Figure 6 is a partial DNA sequence of HIV *gag* (SEQ ID NO: 1), showing inactivation of inhibitory sequences as described in Schwartz, S. *et al.*, *J. Virol.*, *66*(12):7176-7182 (1992).

Figure 7 a plot of the %(G+C) content of wildtype HIV *gagpol* sequences and theoretically codon optimized HIV *gagpol* sequences. The percent of bases, either G or C, was calculated for a 30 nucleotide moving window for the entire length of the *gagpol* gene, and the value plotted versus nucleotide position. Diamonds = HIV *gagpol* sequences; squares = full optimal back-translation for *gag* open reading frame; triangles = full optimal back-translation for *pol* open reading frame; CO = codon optimized.

Figures 8A-8E depict the alignment of the nucleotide sequences and predicted amino acid sequences for the *gag* coding region of a wildtype HIV *gagpol* and a codon optimized HIV *gagpol*. "NL4-3 genbank.SEQ" indicates the nucleotide sequence (SEQ ID NO:2) and predicted amino acid sequence (SEQ ID NO:3) for the *gag* coding region of a wildtype HIV *gagpol*. "pHDMHgpm2.seq" indicates the nucleotide sequence (SEQ ID NO:4) and predicted amino acid sequence (SEQ ID NO:5) for the *gag* coding region

of a codon optimized HIV *gagpol*. The "NL4-3 genbank.SEQ" sequences are publicly available at the NIH GenBank sequence repository (Accesssion No. M19921).

Figures 9A-9L depict the alignment of the nucleotide sequences and predicted amino acid sequences for the *pol* coding region of a wildtype HIV *gagpol* and a codon optimized HIV *gagpol*. "NL4-3 genbank.SEQ" indicates a nucleotide sequence (SEQ ID NO:6) and a predicted amino acid sequence (SEQ ID NO:7) for the *pol* coding region of a wildtype HIV *gagpol* available in the NIH GenBank sequence repository (Accesssion No. M19921). The nucleotide and amino acid sequences for the *pol* coding region available in the GenBank sequence repository contain two sequence errors, which are indicated in Figures 9A-9L with shading. "pNL4-3.seq" indicates the correct nucleotide sequence (SEQ ID NO:8) and predicted amino acid sequence (SEQ ID NO:9) for the *pol* coding region of a wildtype HIV *gagpol*. "pHDMHgpm2.seq" indicates the nucleotide sequence (SEQ ID NO:10) and predicted amino acid sequence (SEQ ID NO:11) for the *pol* coding region of a codon optimized HIV *gagpol*.

Figures 10A-10D depict the DNA sequence (SEQ ID NO:12) for pHDMHgpm2. The CMV enhancer/promoter is at nucleotides 97 to 679, human betaglobin sequences (Bglobin) are at nucleotides 761 to 864, 865 to 1303 and 5710 to 6469 (end of Bglobin is at nucleotdes 6445 to 6469), mRNA sequences are at nucleotides 680 to 778 and 1255 to 5921, SV40 origin of replication is at nucleotides 8796 to 8908, beta-lactamase (bla) coding region is at nucleotides 6709 to 7569, intron sequences are at nucleotides 779 to 1254, the codon optimized *gag* coding region is at nucleotides 1318 to 2820, the codon optimized *pol* coding region is at nucleotides 2619 to 5624 and the poly A site is at nucleotides 5897 to 5921.

Figure 11 is a circular map of plasmid pHDMHgpm2.

## DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to novel packaging cell lines useful for generating viral accessory protein independent lentivirus-derived, particularly HIV-derived,

retroviral vector particles, to construction of such cell lines and to methods of using the accessory protein independent lentivirus-derived retroviral vector particles to introduce DNA of interest into cells (e.g, eukaryotic cells such as animal (particularly mammalian), plant or yeast cells or prokaryotic cells such as bacterial cells). In a particular embodiment, the packaging cell lines of the present invention are stable packaging cell lines.

The cell lines are engineered to express the lentivirus proteins necessary for virus particle formation (gagpol proteins), without containing DNA sequences from lentivirus accessory proteins (tat, vif, vpr, vpu, nef and rev proteins and Rev response element (RRE)). Additionally, no viral sequences (such as cis-acting elements termed constitutive transport elements (CTEs)) will be expressed as RNA of any kind. DNA sequences for lentivirus *gagpol* are codon optimized by extensively mutagenizing the sequences to improve expression and to reduce the risk of recombination between transfer vector sequences and gagpol messenger RNA. This greatly improves the safety of virus preparations generated from these cell lines. In a particular embodiment, the DNA sequences for lentivirus *gagpol* are not codon optimized in the overlap region between the *gag* and *pol* sequences and in cis-acting signals necessary for translation of pol.

Examples of lentiviruses include human immunodeficiency viruses (e.g., HIV-1, HIV-2, HIV-3), bovine lentiviruses (e.g., bovine immunodeficiency viruses, bovine immunodeficiency-like viruses, Jembrana disease viruses), equine lentiviruses (e.g., equine infectious anemia viruses), feline lentiviruses (e.g., feline immunodeficiency viruses, panther lentiviruses, puma lentiviruses), ovine/caprine lentiviruses (e.g., Brazilian caprine lentiviruses, caprine arthritis-encephalitis viruses, Maedi-Visna viruses, Maedi-Visna-like viruses, Maedi-Visna-related viruses, ovine lentiviruses, Visna lentiviruses), Simian AIDS retroviruses (e.g., human T-cell lymphotropic virus type 4), simian immunodeficiency viruses, simian-human immunodeficiency viruses, human lymphotrophic viruses (e.g., type III), simian T-cell lymphotrophic viruses.

In another embodiment, cell lines are engineered to express the HIV proteins necessary for virus particle formation (gagpol proteins), without containing DNA sequences from HIV accessory proteins (tat, vif, vpr, vpu, nef and rev proteins and Rev response element (RRE)). Additionally, no viral sequences (such as cis-acting elements termed constitutive transport elements (CTEs)) will be expressed as RNA of any kind. DNA sequences for a HIV *gagpol* are codon optimized by mutagenesis to improve expression and to reduce the risk of recombination between transfer vector sequences and gagpol messenger RNA. In a particular embodiment, the DNA sequences for HIV *gagpol* are not codon optimized in the overlap region between the *gag* and *pol* sequences and in cis-acting signals necessary for translation of pol.

Alternatively, each of the packaging cell lines described herein can be produced using (1) a nucleotide sequence which comprises a codon optimized gag coding sequence and (2) a nucleotide sequence which comprises a codon optimized pol coding sequence, in place of the nucleotide sequence which comprises a codon optimized gagpol coding sequence. In this embodiment, the gag and pol coding sequences can be completely codon optimized

Benefits of the present invention include the removal of potentially harmful lentivirus accessory proteins and other viral sequences, and the reduction of the risk of recombination to produce replication competent virus.

Packaging cell lines for producing a viral accessory protein independent lentivirus-derived retroviral vector particles comprise a mammalian cell and a retroviral nucleotide sequence comprising a coding sequence for a lentivirus *gagpol* which has been codon optimized. In a particular embodiment the packaging cell lines further comprise a retroviral nucleotide sequence comprising a coding sequence for a heterologous envelope protein. In a second embodiment, the packaging cell lines further comprise a retroviral nucleotide sequence comprising a coding sequence for a heterologous envelope protein and a retroviral nucleotide sequence which comprises a DNA sequence of interest and HIV cis-acting sequences required for packaging, reverse

transcription and integration. In third embodiment, the packaging cell lines further comprise a retroviral nucleotide sequence which comprises a DNA sequence of interest and HIV cis-acting sequences required for packaging, reverse transcription and integration. Alternatively, the packaging cell lines of the present invention comprise a retroviral nucleotide sequence which comprises a codon optimized gag coding sequence and (2) a retroviral nucleotide sequence which comprises a codon optimized pol coding sequence, in place of the retroviral nucleotide sequence which comprises a codon optimized gagpol coding sequence.

The coding sequence(s) for lentivirus *gagpol* which has (have) been codon optimized results in improved expression of the lentivirus gagpol proteins and reduces the risk of recombination between the transfer vector and gagpol messenger RNA. Codon optimization of the coding sequence(s) for lentivirus *gagpol* was obtained by mutagenizing for each particular amino acid residue, specific nucleic acid bases in a codon for the particular amino acid residue to a nucleic acid base which is present in a codon which occurs at a high frequency in genes which are highly expressed for the same amino acid residue. In a particular embodiment, the resulting optimized codon also does not cause introduction of mRNA splicing signals into the codon optimized sequence. Thus, in a particular embodiment, codon optimization of the coding sequence(s) for lentivirus *gagpol* is obtained by mutagenizing for each particular amino acid residue, specific nucleic acid bases in a codon for the particular amino acid residue to a nucleic acid base that is present in a codon which (1) occurs at a high frequency in genes which are highly expressed for the same amino acid residue and (2) does not cause introduction of mRNA splicing signals into the codon optimized sequence. Codon optimization typically results in the removal of nucleic acid base A-rich instability elements.

In a particular embodiment, the coding sequence for a HIV *gagpol* (pNL4-3; available through the AIDS repository, NIH; Adachi *et al.*, *J. Virol.*, 59:284-291 (1986)) has been codon optimized to improve translational efficiency of the HIV gagpol

proteins and reduce the risk of recombination between the transfer vector and HIV gagpol messenger RNA. Two hundred thirty-seven base pairs (237 bp) consisting of the gag pol overlap and cis-acting signals necessary for translation of pol (nucleotides 2583 to 2819 of SEQ ID NO: 12) were not optimized. The HIV *gagpol* sequence obtained using the codon optimization process does not differ at the amino acid level from the wildtype HIV *gagpol* sequence, but differs at the nucleotide level from the HIV *gagpol* sequence. A codon optimized HIV *gag* sequence is shown in Figures 8A-8E (pHDMHgpm2.seq) (SEQ ID NO:4). A codon optimized HIV *pol* sequence is shown in Figures 9A-9L (pHDMHgpm2.seq) (SEQ ID NO:10).

A plasmid comprising DNA sequences which encode codon optimized lentivirus *gagpol* proteins is also referred to herein as a packaging construct. This plasmid includes a promoter which drives the expression of the gagpol proteins, such as the human cytomegalovirus (hCMV) immediate early promoter. This plasmid is defective for the production of the viral envelope and accessory proteins tat, vif, vpr, vpu, nef and rev and the Rev response element (RRE). The packaging construct also does not contain viral sequences which are transcribed into mRNA, such as constitutive transport elements (CTEs).

A packaging construct comprising a codon optimized HIV *gagpol* is depicted in Figure 1 and in Figure 11. Figures 10A-10D depict the DNA sequence (SEQ ID NO:12) for the packaging construct pHDMHgpm2. This packaging construct (pHDMHgpm2) was constructed as follows: Plasmid pMDA.HIVgp mam was generated by chemical synthesis and PCR assembly (which is described in, for example, Stemmer *et al., Gene, 164*:49-53 (1995)) of 215 different oligonucleotides. The DNA sequence for pMDA.HIVgp mam is the same as the DNA sequence for pMDA.HIVgp jtg except for 4.3 kb which was codon optimized using the DNAStar program (LaserGene, Madison, WI). Two hundred thirty-seven base pairs (237 bp) consisting of the gag pol overlap and cis-acting signals necessary for translation of pol (nucleotides 2583 to 2819 of SEQ ID NO: 12) were not optimized due to dual reading

frame constraints. A NsiI site 5' of IN was preserved to aid fusion with wildtype sequences. Several single or double base pair silent mutations were introduced either to prevent potential splice donors and acceptors, or by the synthesis process. pMDA.HIVgp jtg was derived from HIV-1 strain NL4-3. The protease mutation that is present in the NL4-3 NIH GenBank sequence was then repaired (Figure 9B), changing the nucleotide present at position 2948 of SEQ ID NO:12 from a "G" to a "C", thereby producing the codon present at nucleotide positions 2948 to 2950 of SEQ ID NO:12 which encodes an arginine instead of the glycine present in the NL4-3 GenBank amino acid sequence. The resulting plasmid was named pMDHgpmam. The EcoRI-HindIII fragment of pMDHgpmam was inserted into pHDM2b, a high copy version of the pMD vector (Ory, D. *et al.*, *Proc. Natl. Acad. Sci. USA*, 93(21):11400-11406 (1996)), to produce plasmid pHDMHgpm. The sequencing mutation that is present in the RNase domain of the NL4-3 NIH GenBank sequence was repaired (Figure 9H), changing the codon present at nucleotide positions 4724 to 4726 of SEQ ID NO:12 from "GGG" to "AAG", thereby producing a codon encoding a lysine instead of the glycine present in the NL4-3 GenBank amino acid sequence. The resulting plasmid was named pHDMHgpm2. Codon usage frequencies in the codon optimized gagpol open reading frame of the packaging construct pHDMHgpm2 are shown in Figure 2.

As used herein, a heterologous envelope protein permits pseudotyping of particles generated by the packaging construct and includes the G glycoprotein of vesicular stomatitis virus (VSV G) and the amphotropic envelope of the Moloney leukemia virus (MLV). A plasmid comprising a DNA sequence which encodes a heterologous envelope protein is also referred to herein as an envelope coding plasmid.

The terms "mammal" and "mammalian", as used herein, refer to any vertebrate animal, including monotremes, marsupials and placental, that suckle their young and either give birth to living young (eutharian or placental mammals) or are egg-laying (metatharian or nonplacental mammals). Examples of mammalian species include

humans and other primates (e.g., monkeys, chimpanzees), rodents (e.g., rats, mice, guinea pigs) and ruminents (e.g., cows, pigs, horses).

Examples of mammalian cells include human (such as HeLa cells, 293T cells, NIH 3T3 cells), bovine, ovine, porcine, murine (such as embryonic stem cells), rabbit and monkey (such as COS1 cells) cells. The cell may be a non-dividing cell (including hepatocytes, myofibers, hematopoietic stem cells, neurons) or a dividing cell. The cell may be an embryonic cell, bone marrow stem cell or other progenitor cell. Where the cell is a somatic cell, the cell can be, for example, an epithelial cell, fibroblast, smooth muscle cell, blood cell (including a hematopoietic cell, red blood cell, T-cell, B-cell, etc.), tumor cell, cardiac muscle cell, macrophage, dendritic cell, neuronal cell (e.g., a glial cell or astrocyte), or pathogen-infected cell (e.g., those infected by bacteria, viruses, virusoids, parasites, or prions).

Typically, cells isolated from a specific tissue (such as epithelium, fibroblast or hematopoietic cells) are categorized as a "cell-type." The cells can be obtained commercially or from a depository or obtained directly from an animal, such as by biopsy. Alternatively, the cell need not be isolated at all from the animal where, for example, it is desirable to deliver the virus to the animal in gene therapy.

To produce the cell lines of the present invention for producing a viral accessory protein independent lentivirus-derived retroviral vector particles, mammalian host cells are co-transfected with (a) a first plasmid comprising DNA sequence which encode lentivirus *gagpol* proteins, wherein said DNA sequence has been codon optimized by mutagenisis, as described above, to improve expression of the lentivirus gagpol proteins; and (2) a second plasmid comprising a DNA sequence which encodes a heterologous envelope protein, or a retroviral nucleotide sequence which comprises a DNA sequence of interest and lentivirus cis-acting sequences required for packaging, reverse transcription and integration, or both, under conditions appropriate for transfection of the cells.

In a particular embodiment, to produce the cell lines of the present invention for producing viral accessory protein independent HIV-derived retroviral vector particles mammalian host cells were cotransfected with (a) a first plasmid comprising DNA sequence which encode HIV *gagpol* proteins, wherein said DNA sequence has been codon optimized by mutagenisis, as described above, to improve expression of the HIV gagpol proteins; and (2) a second plasmid comprising a DNA sequence which encodes a heterologous envelope protein, or a retroviral nucleotide sequence which comprises a DNA sequence of interest and HIV cis-acting sequences required for packaging, reverse transcription and integration, or both, under conditions appropriate for transfection of the cells.

Virus stocks consisting of viral accessory protein independent lentivirus-derived, particularly HIV-derived, retroviral vector particles of the present invention are produced by maintaining the transfected cells under conditions suitable for virus production (e.g., in an appropriate growth media and for an appropriate period of time). Such conditions, which are not critical to the invention. are generally known in the art. See, e.g., *Sambrook et al., Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor University Press, New York (1989); *Ausubel et al., Current Protocols in Molecular Biology*, John Wiley & Sons, New York (1998); U.S. Patent No. 5,449,614; and U.S. Patent No. 5,460,959, the teachings of which are incorporated herein by reference.

To generate viral accessory protein independent lentivirus-derived retroviral vector particles, mammalian host cells can be co-transfected with (a) a first plasmid comprising DNA sequence which encode lentivirus *gagpol* proteins, wherein said DNA sequence has been codon optimized by mutagenisis, as described above, to improve expression of the lentivirus gagpol proteins; (b) a second plasmid comprising a DNA sequence which encodes a heterologous envelope protein; and (c) a third plasmid comprising a DNA sequence of interest and lentivirus cis-acting sequences required for packaging, reverse transcription and integration. Alternatively, mammalian cells are

transfected with a plasmid comprising a codon optimized DNA sequence encoding a lentivirus gag protein and a plasmid comprising a codon optimized DNA sequence encoding a lentivirus pol protein, in place of the first plasmid comprising a codon optimized DNA sequence encoding both lentivirus gagpol proteins. Alternatively, mammalian host cells are transfected with a plasmid comprising a codon optimized DNA sequence encoding a lentivirus gag protein and a plasmid comprising a codon optimized DNA sequence encoding a lentivirus pol protein, in place of the first plasmid comprising a codon optimized DNA sequence encoding both lentivirus gagpol proteins.

In a particular embodiment, the invention relates to methods of producing viral accessory protein independent HIV-derived retroviral vector particles, comprising co-transfecting mammalian host cells with (a) a first plasmid comprising DNA sequence which encode HIV *gagpol* proteins, wherein said DNA sequence has been codon optimized by mutagenisis, as described above, to improve expression of the HIV gagpol proteins; (b) a second plasmid containing a DNA sequence which encodes a heterologous envelope protein; and (c) a third plasmid comprising a DNA sequence of interest and HIV cis-acting sequences required for packaging, reverse transcription and integration. Alternatively, mammalian host cells are transfected with a plasmid comprising a codon optimized DNA sequence encoding a HIV gag protein and a plasmid comprising a codon optimized DNA sequence encoding a HIV pol protein, in place of the first plasmid comprising a codon optimized DNA sequence encoding both HIV gagpol proteins.

Virus particles produced by the methods described herein, using a codon optimized HIV packaging construct produced as described herein, were compared by Western analysis with virus particles produced as described in Naldini *et al.*, *Science, 272*:263-267 (1996), using the packaging construct plasmid pCMVΔR8.2. Both the immunological reactivity and the proteolytic processing were confirmed to be indistinguishable.

A plasmid comprising a DNA sequence of interest and HIV cis-acting sequences required for packaging, reverse transcription and integration is also referred to herein as a transfer vector. A transfer vector, as used herein, refers to a vehicle which is used to introduce a DNA of interest into a eurkaryotic cell, particularly a mammalian cell. Figure 3 depicts an example of a transfer vector.

DNA sequence of interest, as used herein, include all or a portion of a gene or genes encoding a nucleic acid product whose expression in a cell or a mammal is desired. In a particular embodiment, the nucleic acid product is a heterologous therapeutic protein. Examples of therapeutic proteins include antigens or immunogens, such as a polyvalent vaccine, cytokines, tumor necrosis factor, interferons, interleukins, adenosine deaminase, insulin, T-cell receptors, soluble CD4, growth factors, such as epidermal growth factor, human growth factor, insulin-like growth factors, fibroblast growth factors), blood factors, such as Factor VIII, Factor IX, cytochrome b, glucocerebrosidase, ApoE, ApoC, ApoAI, the LDL receptor, negative selection markers or "suicide proteins", such as thymidine kinase (including the HSV, CMV, VZV TK), anti-angiogenic factors, Fc receptors, plasminogen activators, such as t-PA, u-PA and streptokinase, dopamine, MHC, tumor suppressor genes such as p53 and Rb, monoclonal antibodies or antigen binding fragments thereof, drug resistance genes, ion channels, such as a calcium channel or a potassium channel, adrenergic receptors, hormones (including growth hormones) and anti-cancer agents. In another embodiment, the nucleic acid product is a gene product to be expressed in a cell or a mammal and which product is otherwise defective or absent in the cell or mammal. For example, the nucleic acid product can be a functional gene(s) which is defective or absent in the cell or mammal.

DNA sequence of interest includes DNA sequences (control sequences) which are necessary to drive the expression of the gene or genes. The control sequences are operably linked to the gene. The term "operably linked", as used herein, is defined to mean that the gene is linked to control sequences in a manner which allows expression

of the gene (or the nucleic acid sequence). Generally, operably linked means contiguous.

Control sequences include a transcriptional promoter, an optional operator sequence to control transcription, a sequence encoding suitable mRNA ribosomal binding sites and sequences which control termination of transcription and translation. In a particular embodiment, a recombinant gene encoding a desired nucleic acid product can be placed under the regulatory control of a promoter which can be induced or repressed, thereby offering a greater degree of control with respect to the level of the product produced.

As used herein, the term "promoter" refers to a sequence of DNA, usually upstream (5') of the coding region of a structural gene, which controls the expression of the coding region by providing recognition and binding sites for RNA polymerase and other factors which may be required for initiation of transcription. Suitable promoters are well known in the art. Exemplary promoters include the SV40, CMV and human elongation factor (EFI) promoters. Other suitable promoters are readily available in the art (see, e.g., Ausubel *et al., Current Protocols in Molecular Biology,* John Wiley & Sons, Inc., New York (1998); Sambrook *et al., Molecular Cloning: A Laboratory Manual*, 2nd edition, Cold Spring Harbor University Press, New York (1989); and U.S. Patent No. 5,681,735).

A DNA sequence of interest can be isolated from nature, modified from native sequences or manufactured *de novo*, as described in, for example, Ausubel *et al., Current Protocols in Molecular Biology*, John Wiley & Sons, New York (1998); and Sambrook *et al., Molecular Cloning: A Laboratory Manual*, 2nd edition, Cold Spring Harbor University Press, New York. (1989). DNA sequences can be isolated and fused together by methods known in the art, such as exploiting and manufacturing compatible cloning or restriction sites.

The packaging cell lines and viral particles of the present invention can be used, in vitro, in vivo and ex vivo, to introduce DNA of interest into a eukaryotic cell (e.g., a

mammalian cell) or a mammal (e.g., a human or other mammal or vertebrate). The cells can be obtained commercially or from a depository or obtained directly from a mammal, such as by biopsy. The cells can be obtained from a mammal to whom they will be returned or from another/different mammal of the same or different species. For example, using the packaging cell lines or viral particles of the present invention, DNA of interest can be introduced into nonhuman cells, such as pig cells, which are then introduced into a human. Alternatively, the cell need not be isolated from the mammal where, for example, it is desirable to deliver vial particles of the present invention to the mammal in gene therapy.

*Ex vivo* therapy has been described, for example, in Kasid *et al., Proc. Natl. Acad. Sci. USA, 87*:473 (1990); Rosenberg *et al., N. Engl. J. Med., 323*:570 (1990); Williams *et al., Nature, 310*:476 (1984); Dick *et al., Cell, 42*:71 (1985); Keller *et al., Nature, 318*:149 (1985); and Anderson *et al.*, United States Patent No. 5,399,346.

Methods for administering (introducing) viral particles directly to a mammal are generally known to those practiced in the art. For example, modes of administration include parenteral, injection, mucosal, systemic, implant, intraperitoneal, oral, intradermal, transdermal (e.g., in slow release polymers), intramuscular, intravenous including infusion and/or bolus injection, subcutaneous, topical, epidural, etc. Viral particles of the present invention can, preferably, be administered in a pharmaceutically acceptable carrier, such as saline, sterile water, Ringer's solution, and isotonic sodium chloride solution.

The dosage of a viral particle of the present invention administered to a mammal, including frequency of administration, will vary depending upon a variety of factors, including mode and route of administration; size, age, sex, health, body weight and diet of the recipient mammal; nature and extent of symptoms of the disease or disorder being treated; kind of concurrent treatment, frequency of treatment, and the effect desired.

The teachings of all the articles, patents, patent applications and GenBank sequences cited herein are incorporated by reference in their entirety.

While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the spirit and scope of the invention as defined by the appended claims.

## CLAIMS

What is claimed is:

1. A packaging cell line for producing a viral accessory protein independent HIV-derived retroviral vector particle comprising:
   a) a mammalian cell;
   b) a first retroviral nucleotide sequence in the cell which comprises a coding sequence for a HIV *gagpol*, wherein said coding sequence has been mutagenized to improve expression of the HIV gagpol proteins;
   c) a second retroviral nucleotide sequence in the cell which comprises the coding sequence for a heterologous envelope protein; and
   d) a third retroviral nucleotide sequence in the cell which comprises a DNA sequence of interest and HIV cis-acting sequences required for packaging, reverse transcription and integration.

2. A packaging cell line of Claim 1 wherein the heterologous envelope protein is the G glycoprotein of vesicular stomatitis virus (VSV G).

3. A packaging cell line of Claim 1 wherein the heterologous envelope protein is the amphotropic envelope of the Moloney leukemia virus.

4. A packaging cell line of Claim 1 wherein the DNA sequence of interest encodes a heterologous therapeutic protein.

5. A packaging cell line comprising:
   a) a mammalian cell;

b) a first retroviral nucleotide sequence in the cell which comprises a coding sequence for a HIV *gagpol*, wherein said coding sequence has been mutagenized to improve expression of the HIV gagpol proteins; and

c) a second retroviral nucleotide sequence in the cell which comprises a DNA sequence of interest and HIV cis-acting sequences required for packaging, reverse transcription and integration.

6. A packaging cell line of Claim 5 wherein the DNA sequence of interest encodes a heterologous therapeutic protein.

7. A packaging cell line comprising:

a) a mammalian cell;

b) a first retroviral nucleotide sequence in the cell which comprises a coding sequence for a HIV *gagpol*, wherein said coding sequence has been mutagenized to improve expression of the HIV gagpol proteins; and

c) a second retroviral nucleotide sequence in the cell which comprises the coding sequence for a heterologous envelope protein.

8. A method of producing a packaging cell line for producing a viral accessory protein independent HIV-derived retroviral vector particle, comprising co-transfecting mammalian host cells with:

a) a first plasmid comprising a DNA sequence which encodes HIV *gagpol* proteins, wherein said DNA sequence has been mutagenized to improve expression of the HIV gag and pol proteins;

b) a second plasmid comprising a DNA sequence which encodes a heterologous envelope protein; and

c) a third plasmid comprising a DNA sequence of interest and HIV cis-acting sequences required for packaging, reverse transcription and integration.

9. A method of Claim 8 wherein the heterologous envelope protein is the G glycoprotein of vesicular stomatitis virus (VSV G).

10. A method of Claim 8 wherein the heterologous envelope protein is the amphotropic envelope of the Moloney leukemia virus.

11. A method of Claim 8 wherein the DNA sequence of interest is a heterologous therapeutic protein.

12. A method of producing a viral accessory protein independent HIV-derived retroviral vector particle comprising co-transfecting mammalian host cells with:

a) a first plasmid comprising a DNA sequence which encodes HIV *gagpol* proteins, wherein said DNA sequence has been mutagenized to improve expression of the HIV gagpol proteins;

b) a second plasmid comprising a DNA sequence which encodes a heterologous envelope protein; and

c) a third plasmid comprising a DNA sequence of interest and HIV cis-acting sequences required for packaging, reverse transcription and integration.

13. A method of Claim 12 wherein the heterologous envelope protein is the G glycoprotein of vesicular stomatitis virus (VSV G).

14. A method of Claim 12 wherein the heterologous envelope protein is the amphotropic envelope of the Moloney leukemia virus.

15. A method of Claim 12 wherein the DNA sequence of interest encodes a heterologous therapeutic protein.

16. A packaging cell line for producing a viral accessory protein independent lentivirus-derived retroviral vector particle comprising:

a) a mammalian cell;

b) a first retroviral nucleotide sequence in the cell which comprises a coding sequence for a lentivirus *gagpol*, wherein said coding sequence has been mutagenized to improve expression of the lentivirus gagpol proteins;

c) a second retroviral nucleotide sequence in the cell which comprises the coding sequence for a heterologous envelope protein; and

d) a third retroviral nucleotide sequence in the cell which comprises a DNA sequence of interest and lentivirus cis-acting sequences required for packaging, reverse transcription and integration.

17. A packaging cell line of Claim 16 wherein the heterologous envelope protein is the G glycoprotein of vesicular stomatitis virus (VSV G).

18. A packaging cell line of Claim 16 wherein the heterologous envelope protein is the amphotropic envelope of the Moloney leukemia virus.

19. A packaging cell line of Claim 16 wherein the DNA sequence of interest encodes a heterologous therapeutic protein.

20. A packaging cell line comprising:

a) a mammalian cell;

b) a first retroviral nucleotide sequence in the cell which comprises a coding sequence for lentivirus *gagpol*, wherein said coding sequence ~~has~~ been mutagenized to improve expression of the lentivirus gagpol proteins; and

c) a second retroviral nucleotide sequence in the cell which comprises a DNA sequence of interest and lentivirus cis-acting sequences required for packaging, reverse transcription and integration.

21. A packaging cell line of Claim 20 wherein the DNA sequence of interest encodes a heterologous therapeutic protein.

22. A packaging cell line comprising:

a) a mammalian cell;

b) a first retroviral nucleotide sequence in the cell which comprises a coding sequence for lentivirus *gagpol*, wherein said coding sequence has been mutagenized to improve expression of the lentivirus gagpol proteins; and

c) a second retroviral nucleotide sequence in the cell which comprises the coding sequence for a heterologous envelope protein.

23. A method of producing a packaging cell line for producing a viral accessory protein independent lentivirus-derived retroviral vector particle, comprising co-transfecting mammalian host cells with:

a) a first plasmid comprising a DNA sequence which encodes lentivirus *gagpol* proteins, wherein said DNA sequence has been mutagenized to improve expression of the lentivirus gag and pol proteins;

b) a second plasmid comprising a DNA sequence which encodes a heterologous envelope protein; and

c) a third plasmid comprising a DNA sequence of interest and lentivirus cis-acting sequences required for packaging, reverse transcription and integration.

24. A method of Claim 23 wherein the heterologous envelope protein is the G glycoprotein of vesicular stomatitis virus (VSV G).

25. A method of Claim 23 wherein the heterologous envelope protein is the amphotropic envelope of the Moloney leukemia virus.

26. A method of Claim 23 wherein the DNA sequence of interest is a heterologous therapeutic protein.

27. A method of producing a viral accessory protein independent lentivirus-derived retroviral vector particle comprising co-transfecting mammalian host cells with:

a) a first plasmid comprising a DNA sequence which encodes lentivirus *gagpol* proteins, wherein said DNA sequence has been mutagenized to improve expression of the lentivirus gagpol proteins;

b) a second plasmid comprising a DNA sequence which encodes a heterologous envelope protein; and

c) a third plasmid comprising a DNA sequence of interest and lentivirus cis-acting sequences required for packaging, reverse transcription and integration.

28. A method of Claim 27 wherein the heterologous envelope protein is the G glycoprotein of vesicular stomatitis virus (VSV G).

29. A method of Claim 27 wherein the heterologous envelope protein is the amphotropic envelope of the Moloney leukemia virus.

30. A method of Claim 27 wherein the DNA sequence of interest encodes a heterologous therapeutic protein.

31. A viral accessory protein independent HIV-derived retroviral vector particle produced by the method comprising co-transfecting mammalian host cells with:
    a) a first plasmid comprising a DNA sequence which encodes HIV *gagpol* proteins, wherein said DNA sequence has been mutagenized to improve expression of the HIV gagpol proteins;
    b) a second plasmid comprising a DNA sequence which encodes a heterologous envelope protein; and
    c) a third plasmid comprising a DNA sequence of interest and HIV cis-acting sequences required for packaging, reverse transcription and integration.

32. A method of Claim 31 wherein the heterologous envelope protein is the G glycoprotein of vesicular stomatitis virus (VSV G).

33. A method of Claim 31 wherein the heterologous envelope protein is the amphotropic envelope of the Moloney leukemia virus.

34. A method of Claim 31 wherein the DNA sequence of interest encodes a heterologous therapeutic protein.

35. A viral accessory protein independent lentivirus-derived retroviral vector particle produced by the method comprising co-transfecting mammalian host cells with:

a) a first plasmid comprising a DNA sequence which encodes lentivirus *gagpol* proteins, wherein said DNA sequence has been mutagenized to improve expression of the lentivirus gagpol proteins;

b) a second plasmid comprising a DNA sequence which encodes a heterologous envelope protein; and

c) a third plasmid comprising a DNA sequence of interest and lentivirus cis-acting sequences required for packaging, reverse transcription and integration.

36. A method of Claim 35 wherein the heterologous envelope protein is the G glycoprotein of vesicular stomatitis virus (VSV G).

37. A method of Claim 35 wherein the heterologous envelope protein is the amphotropic envelope of the Moloney leukemia virus.

38. A method of Claim 35 wherein the DNA sequence of interest encodes a heterologous therapeutic protein.

39. Isolated DNA encoding a codon optimized HIV *gagpol*.

40. Isolated DNA encoding a codon optimized HIV *gag*.

41. Isolated DNA of Claim 40 comprising the nucleotide sequence of SEQ ID NO:4.

42. Isolated DNA encoding a codon optimized HIV *pol*.

43. Isolated DNA of Claim 42 comprising the nucleotide sequence of SEQ ID NO:10.

44. A method of introducing a DNA sequence of interest into a mammal comprising introducing into said mammal a viral accessory protein independent HIV-derived retroviral vector particle comprising the DNA sequence of interest.

45. The method of Claim 44 wherein the mammal is a human.

46. The method of Claim 44 wherein the DNA sequence of interest encodes a heterologous therapeutic protein.

47. A method of introducing a DNA sequence of interest into a mammal comprising the steps of:

a) introducing into cells a viral accessory protein independent HIV-derived retroviral vector particle comprising the DNA sequence of interest; and

b) returning the cells obtained in step a) to the mammal.

48. The method of Claim 47 wherein the mammal is a human.

49. The method of Claim 47 wherein the DNA sequence of interest is a heterologous therapeutic protein.

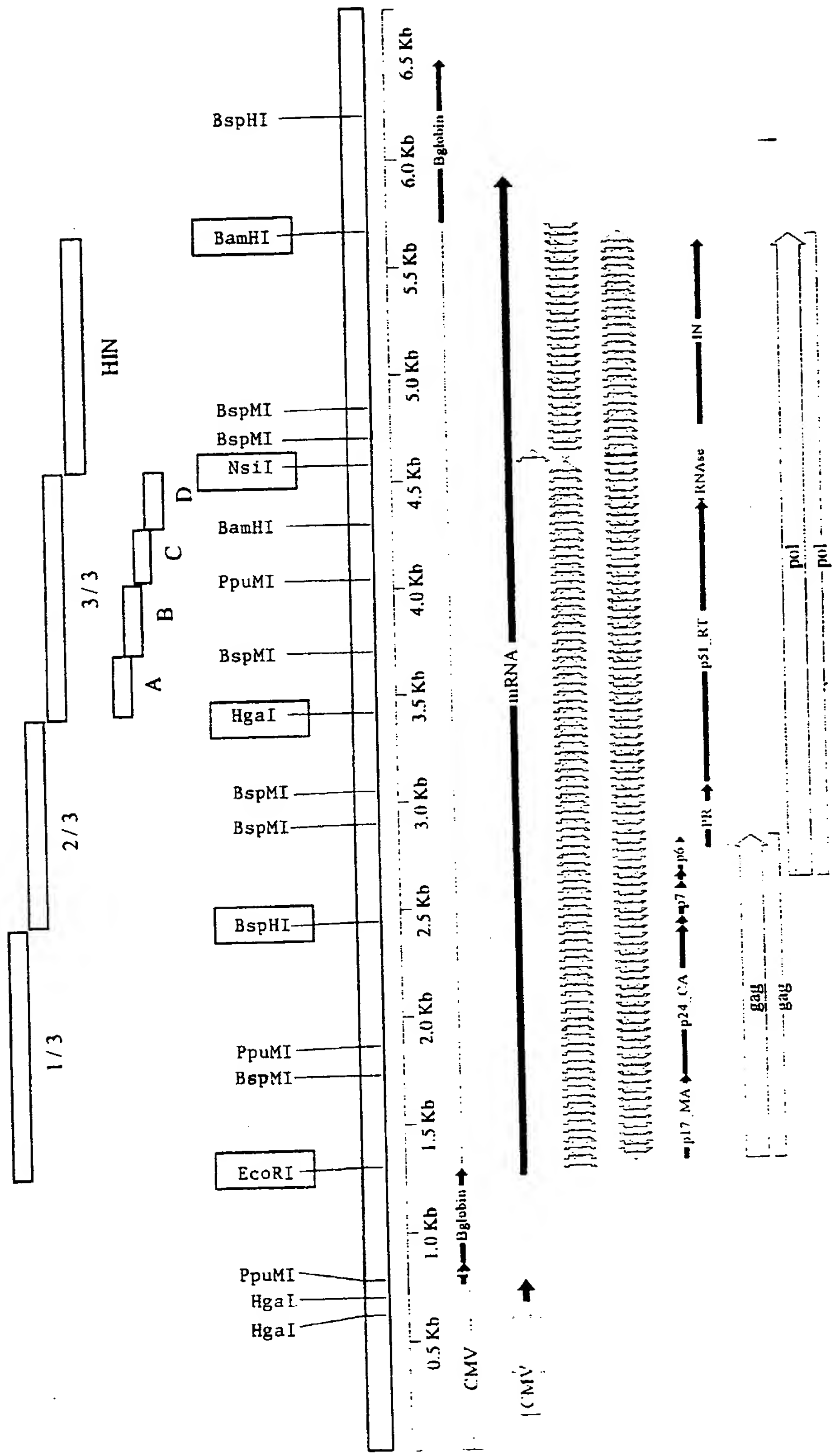
BspHI
BamHI
HIN
BspMI
BspMI
NsiI
D
BamHI
C
3/3
PpuMI
B
BspMI
A
HgaI
BspMI
BspMI
2/3
BspHI
1/3
PpuMI
BspMI
EcoRI
PpuMI
HgaI
HgaI
6.5 Kb
6.0 Kb
5.5 Kb
5.0 Kb
4.5 Kb
4.0 Kb
3.5 Kb
3.0 Kb
2.5 Kb
2.0 Kb
1.5 Kb
1.0 Kb
0.5 Kb
Bglobin
Bglobin
CMV
mRNA
CMV
IN
RNAse
p51 RT
PR
p6
p7
p24 CA
p17 MA
pol
pol
gag
gag

Fig. 1

## Codon Usage Frequencies

| Amino Acid | pNL4-3 gagpol | mam | Amino Acid | pNL4-3 gagpol | mam | Amino Acid | pNL4-3 gagpol | mam |
|---|---|---|---|---|---|---|---|---|
| gca Ala(A) | 58 | 13 | gga Gly(G) | 55 | 14 | cca Pro(P) | 53 | 16 |
| gcc Ala(A) | 23 | 53 | ggc Gly(G) | 12 | 50 | ccc Pro(P) | 17 | 48 |
| gcg Ala(A) | 5 | 17 | ggg Gly(G) | 27 | 24 | ccg Pro(P) | 2 | 17 |
| gcu Ala(A) | 14 | 17 | ggu Gly(G) | 6 | 12 | ccu Pro(P) | 27 | 19 |
| aga Arg(R) | 63 | 10 | cac Hi s(H) | 24 | 79 | agc Ser (S) | 29 | 34 |
| agg Arg(R) | 30 | 18 | cau Hi s(H) | 76 | 21 | agu Ser (S) | 26 | 10 |
| cga Arg(R) | 4 | 6 | | | | uca Ser (S) | 26 | 5 |
| cgc Arg(R) | 0 | 37 | aua Ile(I) | 57 | 5 | ucc Ser (S) | 7 | 28 |
| cgg Arg(R) | 3 | 21 | auc Ile(I) | 17 | 77 | ucg Ser (S) | 4 | 9 |
| cgu Arg(R) | 0 | 7 | auu Ile(I) | 26 | 18 | ucu Ser (S) | 6 | 13 |
| aac Asn(N) | 27 | 78 | cua Leu(L) | 15 | 3 | aca Thr (T) | 52 | 14 |
| aau Asn(N) | 73 | 22 | cuc Leu(L) | 10 | 26 | acc Thr (T) | 18 | 57 |
| gac Asp(D) | 40 | 75 | cug Leu(L) | 11 | 58 | acg Thr (T) | 1 | 15 |
| gau Asp(D) | 60 | 25 | cuu Leu(L) | 11 | 5 | acu Thr (T) | 29 | 14 |
| ugc Cys (C) | 14 | 68 | uua Leu(L) | 40 | 2 | ugg Trp(W) | 100 | 100 |
| ugu Cys (C) | 26 | 32 | uug Leu(L) | 13 | 6 | | | |
| caa Gln(Q) | 56 | 12 | aaa Lys (K) | 69 | 18 | uac Tyr (Y) | 26 | 74 |
| cag Gln(Q) | 44 | 88 | aag Lys (K) | 31 | 82 | uau Tyr (Y) | 74 | 26 |
| | | | aug Met (M) | 100 | 100 | gua Val (V) | 58 | 5 |
| gaa Glu(E) | 70 | 25 | | | | guc Val (V) | 13 | 25 |
| gag Glu(E) | 30 | 75 | uuc Phe (F) | 40 | 80 | gug Val (V) | 16 | 64 |
| | | | uuu Phe (F) | 60 | 20 | guu Val (V) | 14 | 7 |

Fig. 2

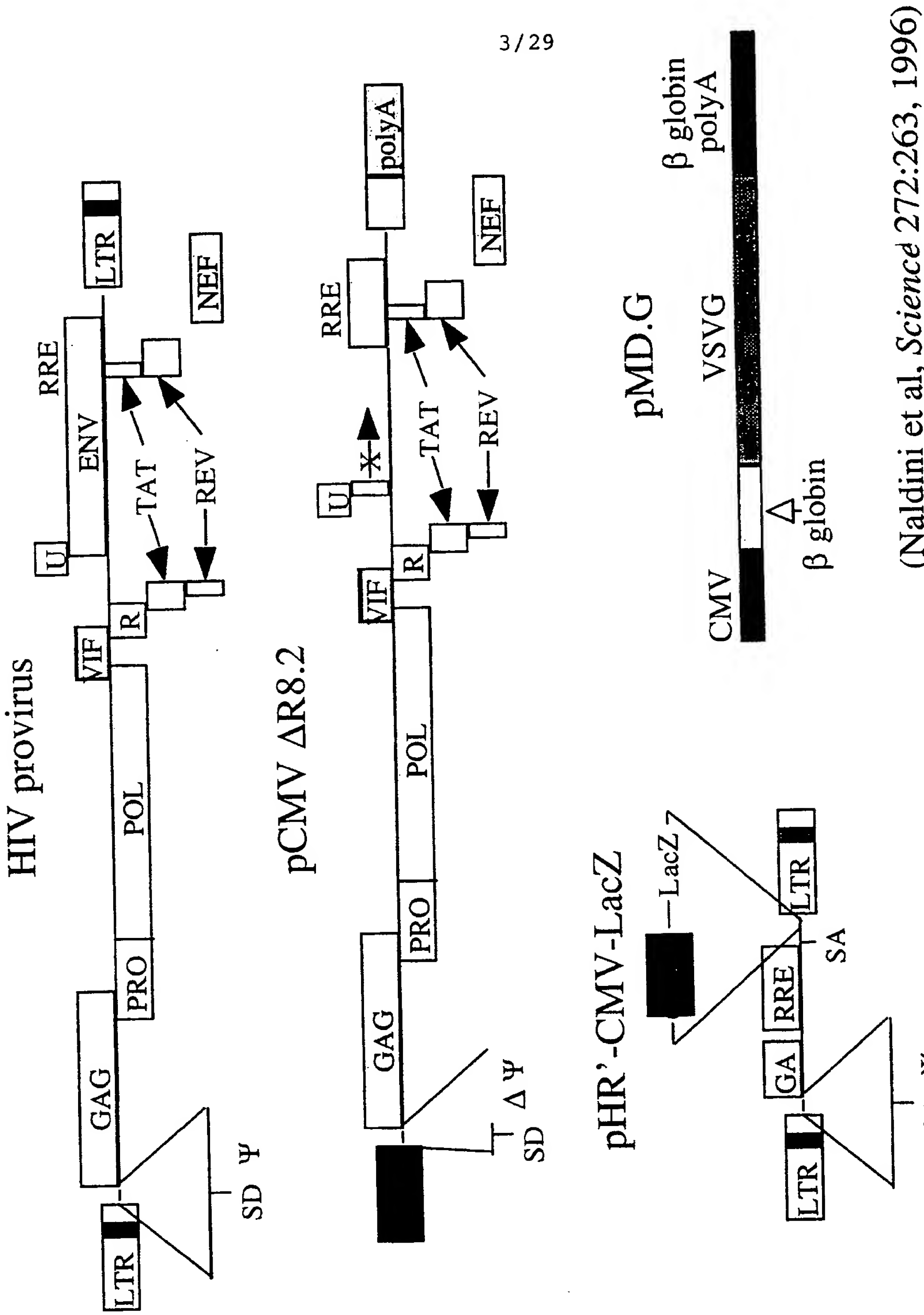
HIV provirus
LTR
GAG
PRO
POL
VIF
R
U
ENV
RRE
TAT
REV
NEF
SD Ψ
pCMV ΔR8.2
polyA
SD
Δ Ψ
pHR'-CMV-LacZ
LacZ
GA
SA
pMD.G
CMV
β globin
VSVG
β globin polyA
(Naldini et al, Science 272:263, 1996)

Fig. 3

# Rev

- Regulates HIV gene expression by promoting cytoplasmic levels of unspliced and singly spliced mRNAs
- Postulated to affect splicing, stability, transport, and translation

Fig. 4

# Codon Optimization of HIV *gagpol*

- Remove A-rich instability elements
- Improve translational efficiency
- Reduce risk of recombination with transfer vector

Fig. 5

# Inactivation of Inhibitory Sequences in *gag*

**Schwartz, S., *et al.***

336

atg ggt gcg aga gcg tca gta tta agc ggg gga gaa tta gat cga tgg gaa aaa att cgg

396 **M1**

tta agg cca ggg gga aag aaa aaa tat aaa tta aaa cat ata gta tgg gca agc agg gag

456 G G C GC G C C

cta gaa cga ttc gca gtt aat cct ggc ctg tta gaa aca tca gaa ggc tgt aga caa ata

516 **M2**

ctg gga cag cta caa cca tcc ctt cag aca gga tca gaa gaa ctt aga tca tta tat aat

576 **M3** G G C C C C

aca gta gca acc ctc tat tgt gtg cat caa agg ata gag ata aaa gac acc aag gaa gct

C GC C C G

636 **M4**

tta gac aag ata gag gaa gag caa aac aaa agt aag aaa aaa gca cag caa gca gca gct

696 GTCC G G C G

gac aca gga cac agc aat cag gtc agc caa aat tac

**Fig. 6**

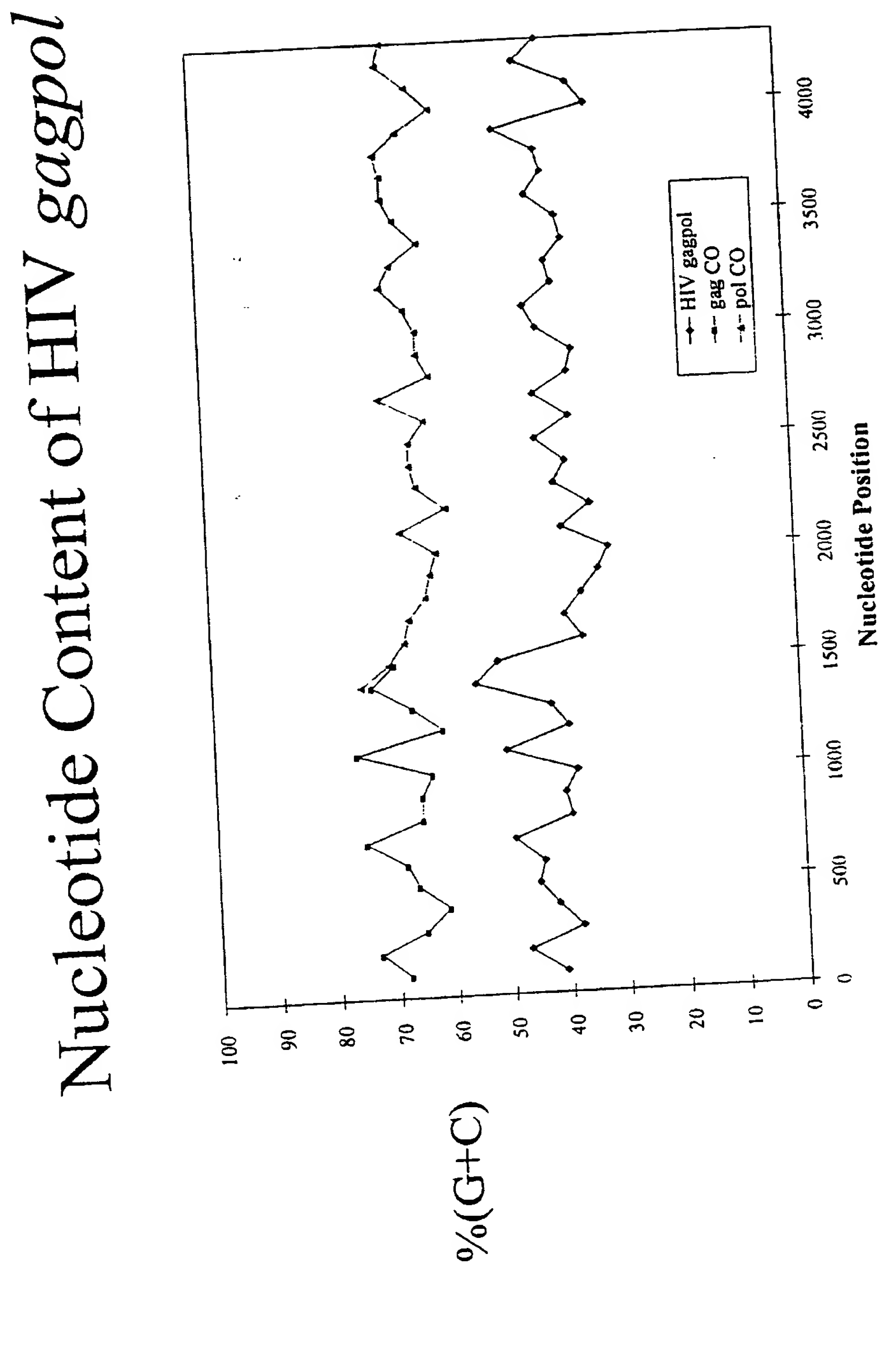
Nucleotide Content of HIV gagpol
%(G+C)
100
90
80
70
60
50
40
30
20
10
0
0
500
1000
1500
2000
2500
3000
3500
4000
Nucleotide Position
HIV gagpol
gag CO
pol CO

Fig. 7

Alignment Report of Codon optimization (gag).MEG, using Clustal method with PAM250 residue weight table.

810

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 792 | M | G | A | R | A | S | V | L | S | G | G | E | L | D | K | NL4-3 genbank.SEQ |
| 792 | ATG | GGT | GCG | AGA | GCG | TCG | GTA | TTA | AGC | GGG | GGA | GAA | TTA | GAT | AAA | |
| 1319 | M | G | A | R | A | S | V | L | S | G | G | E | L | D | K | pHDMHgpm2.seq |
| 1319 | ATG | GGC | GCC | CGC | GCC | TCC | GTG | CTG | TCC | GGC | GGC | GAG | CTG | GAC | AAG | |

840 870

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 837 | W | E | K | I | R | L | R | P | G | G | K | K | Q | Y | K | NL4-3 genbank.SEQ |
| 837 | TGG | GAA | AAA | ATT | CGG | TTA | AGG | CCA | GGG | GGA | AAG | AAA | CAA | TAT | AAA | |
| 1364 | W | E | K | I | R | L | R | P | G | G | K | K | Q | Y | K | pHDMHgpm2.seq |
| 1364 | TGG | GAG | AAG | ATC | CGC | CTG | CGC | CCC | GGC | GGC | AAG | AAG | CAG | TAC | AAG | |

900

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 882 | L | K | H | I | V | W | A | S | R | E | L | E | R | F | A | NL4-3 genbank.SEQ |
| 882 | CTA | AAA | CAT | ATA | GTA | TGG | GCA | AGC | AGG | GAG | CTA | GAA | CGA | TTC | GCA | |
| 1409 | L | K | H | I | V | W | A | S | R | E | L | E | R | F | A | pHDMHgpm2.seq |
| 1409 | CTG | AAG | CAC | ATC | GTG | TGG | GCC | TCC | CGC | GAG | CTG | GAG | CGC | TTC | GCC | |

930 960

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 927 | V | N | P | G | L | L | E | T | S | E | G | C | R | Q | I | NL4-3 genbank.SEQ |
| 927 | GTT | AAT | CCT | GGC | CTT | TTA | GAG | ACA | TCA | GAA | GGC | TGT | AGA | CAA | ATA | |
| 1454 | V | N | P | G | L | L | E | T | S | E | G | C | R | Q | I | pHDMHgpm2.seq |
| 1454 | GTG | AAC | CCC | GGC | CTG | CTG | GAG | ACC | TCC | GAG | GGC | TGC | CGC | CAG | ATC | |

990

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 972 | L | G | Q | L | Q | P | S | L | Q | T | G | S | E | E | L | NL4-3 genbank.SEQ |
| 972 | CTG | GGA | CAG | CTA | CAA | CCA | TCC | CTT | CAG | ACA | GGA | TCA | GAA | GAA | CTT | |
| 1499 | L | G | Q | L | Q | P | S | L | Q | T | G | S | E | E | L | pHDMHgpm2.seq |
| 1499 | CTG | GGC | CAG | CTG | CAG | CCC | TCC | CTG | CAA | ACC | GGC | TCC | GAG | GAG | CTG | |

1020 1050

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1017 | R | S | L | Y | N | T | I | A | V | L | Y | C | V | H | Q | NL4-3 genbank.SEQ |
| 1017 | AGA | TCA | TTA | TAT | AAT | ACA | ATA | GCA | GTC | CTC | TAT | TGT | GTG | CAT | CAA | |
| 1544 | R | S | L | Y | N | T | I | A | V | L | Y | C | V | H | Q | pHDMHgpm2.seq |
| 1544 | CGC | TCC | CTG | TAC | AAC | ACC | ATC | GCC | GTG | CTG | TAC | TGC | GTG | CAC | CAG | |

1080

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1062 | R | I | D | V | K | D | T | K | E | A | L | D | K | I | E | NL4-3 genbank.SEQ |
| 1062 | AGG | ATA | GAT | GTA | AAA | GAC | ACC | AAG | GAA | GCC | TTA | GAT | AAG | ATA | GAG | |
| 1589 | R | I | D | V | K | D | T | K | E | A | L | D | K | I | E | pHDMHgpm2.seq |
| 1589 | CGC | ATC | GAC | GTG | AAG | GAC | ACC | AAG | GAG | GCC | CTG | GAC | AAG | ATC | GAG | |

1110 1140

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1107 | E | E | Q | N | K | S | K | K | K | A | Q | Q | A | A | A | NL4-3 genbank.SEQ |
| 1107 | GAA | GAG | CAA | AAC | AAA | AGT | AAG | AAA | AAG | GCA | CAG | CAA | GCA | GCA | GCT | |
| 1634 | E | E | Q | N | K | S | K | K | K | A | Q | Q | A | A | A | pHDMHgpm2.seq |
| 1634 | GAG | GAG | CAG | AAC | AAG | TCC | AAG | AAG | AAG | GCC | CAG | CAG | GCC | GCC | GCC | |

Fig. 8A

Alignment Report of Codon optimization (gag).MEG, using Clustal method with PAM250 residue weight table.

```
                            1170
1152  D   T   G   N   N   S   Q   V   S   Q   N   Y   P   I   V    NL4-3 genbank.SEQ
1152  GAC ACA GGA AAC AAC AGC CAG GTC AGC CAA AAT TAC CCT ATA GTG
1679  D   T   G   N   N   S   Q   V   S   Q   N   Y   P   I   V    pHDMHgpm2.seq
1679  GAC ACC GGC AAC AAC TCC CAG GTG TCC CAG AAC TAC CCC ATC GTG

        1200                                      1230
1197  Q   N   L   Q   G   Q   M   V   H   Q   A   I   S   P   R    NL4-3 genbank.SEQ
1197  CAG AAC CTC CAG GGG CAA ATG GTA CAT CAG GCC ATA TCA CCT AGA
1724  Q   N   L   Q   G   Q   M   V   H   Q   A   I   S   P   R    pHDMHgpm2.seq
1724  CAG AAC CTG CAG GGC CAG ATG GTG CAC CAG GCC ATC TCC CCC CGC

                            1260
1242  T   L   N   A   W   V   K   V   V   E   E   K   A   F   S    NL4-3 genbank.SEQ
1242  ACT TTA AAT GCA TGG GTA AAA GTA GTA GAA GAG AAG GCT TTC AGC
1769  T   L   N   A   W   V   K   V   V   E   E   K   A   F   S    pHDMHgpm2.seq
1769  ACC CTG AAC GCC TGG GTG AAG GTG GTG GAG GAG AAG GCC TTC TCC

        1290                                      1320
1287  P   E   V   I   P   M   F   S   A   L   S   E   G   A   T    NL4-3 genbank.SEQ
1287  CCA GAA GTA ATA CCC ATG TTT TCA GCA TTA TCA GAA GGA GCC ACC
1814  P   E   V   I   P   M   F   S   A   L   S   E   G   A   T    pHDMHgpm2.seq
1814  CCC GAA GTC ATC CCC ATG TTC TCC GCC CTG TCC GAG GGC GCC ACC

                            1350
1332  P   Q   D   L   N   T   M   L   N   T   V   G   G   H   Q    NL4-3 genbank.SEQ
1332  CCA CAA GAT TTA AAT ACC ATG CTA AAC ACA GTG GGG GGA CAT CAA
1859  P   Q   D   L   N   T   M   L   N   T   V   G   G   H   Q    pHDMHgpm2.seq
1859  CCC CAG GAC CTG AAC ACC ATG CTG AAC ACC GTG GGC GGC CAC CAG

        1380                                      1410
1377  A   A   M   Q   M   L   K   E   T   I   N   E   E   A   A    NL4-3 genbank.SEQ
1377  GCA GCC ATG CAA ATG TTA AAA GAG ACC ATC AAT GAG GAA GCT GCA
1904  A   A   M   Q   M   L   K   E   T   I   N   E   E   A   A    pHDMHgpm2.seq
1904  GCC GCC ATG CAG ATG CTG AAG GAG ACC ATC AAC GAG GAG GCC GCC

                            1440
1422  E   W   D   R   L   H   P   V   H   A   G   P   I   A   P    NL4-3 genbank.SEQ
1422  GAA TGG GAT AGA TTG CAT CCA GTG CAT GCA GGG CCT ATT GCA CCA
1949  E   W   D   R   L   H   P   V   H   A   G   P   I   A   P    pHDMHgpm2.seq
1949  GAG TGG GAC CGC CTG CAC CCC GTG CAC GCC GGC CCC ATC GCC CCC

        1470                                      1500
1467  G   Q   M   R   E   P   R   G   S   D   I   A   G   T   T    NL4-3 genbank.SEQ
1467  GGC CAG ATG AGA GAA CCA AGG GGA AGT GAC ATA GCA GGA ACT ACT
1994  G   Q   M   R   E   P   R   G   S   D   I   A   G   T   T    pHDMHgpm2.seq
1994  GGC CAG ATG CGC GAG CCC CGC GGC TCC GAC ATC GCC GGC ACC ACC
```

Fig. 8B

Alignment Report of Codon optimization (gag).MEG, using Clustal method with PAM250 residue weight table.

```
                              1530
1512  S   T   L   Q   E   Q   I   G   W   M   T   H   N   P   P   NL4-3 genbank.SEQ
1512 AGT ACC CTT CAG GAA CAA ATA GGA TGG ATG ACA CAT AAT CCA CCT
2039  S   T   L   Q   E   Q   I   G   W   M   T   H   N   P   P   pHDMHgpm2.seq
2039 TCC ACC CTG CAA GAG CAG ATC GGC TGG ATG ACC CAC AAC CCC CCC

        1560                                      1590
1557  I   P   V   G   E   I   Y   K   R   W   I   I   L   G   L   NL4-3 genbank.SEQ
1557 ATC CCA GTA GGA GAA ATC TAT AAA AGA TGG ATA ATC CTG GGA TTA
2084  I   P   V   G   E   I   Y   K   R   W   I   I   L   G   L   pHDMHgpm2.seq
2084 ATC CCC GTG GGC GAG ATC TAC AAG CGC TGG ATC ATC CTG GGC CTG

                              1620
1602  N   K   I   V   R   M   Y   S   P   T   S   I   L   D   I   NL4-3 genbank.SEQ
1602 AAT AAA ATA GTA AGA ATG TAT AGC CCT ACC AGC ATT CTG GAC ATA
2129  N   K   I   V   R   M   Y   S   P   T   S   I   L   D   I   pHDMHgpm2.seq
2129 AAC AAG ATC GTG CGC ATG TAC TCC CCC ACC TCC ATC CTG GAC ATC

        1650                                      1680
1647  R   Q   G   P   K   E   P   F   R   D   Y   V   D   R   F   NL4-3 genbank.SEQ
1647 AGA CAA GGA CCA AAG GAA CCC TTT AGA GAC TAT GTA GAC CGA TTC
2174  R   Q   G   P   K   E   P   F   R   D   Y   V   D   R   F   pHDMHgpm2.seq
2174 CGC CAG GGC CCC AAG GAG CCC TTC CGC GAC TAC GTG GAC CGC TTC

                              1710
1692  Y   K   T   L   R   A   E   Q   A   S   Q   E   V   K   N   NL4-3 genbank.SEQ
1692 TAT AAA ACT CTA AGA GCC GAG CAA GCT TCA CAA GAG GTA AAA AAT
2219  Y   K   T   L   R   A   E   Q   A   S   Q   E   V   K   N   pHDMHgpm2.seq
2219 TAC AAG ACC CTG CGC GCC GAG CAG GCC TCC CAG GAG GTA AAG AAC

        1740                                      1770
1737  W   M   T   E   T   L   L   V   Q   N   A   N   P   D   C   NL4-3 genbank.SEQ
1737 TGG ATG ACA GAA ACC TTG TTG GTC CAA AAT GCG AAC CCA GAT TGT
2264  W   M   T   E   T   L   L   V   Q   N   A   N   P   D   C   pHDMHgpm2.seq
2264 TGG ATG ACC GAG ACC CTG CTG GTG CAG AAC GCC AAC CCC GAC TGC

                              1800
1782  K   T   I   L   K   A   L   G   P   G   A   T   L   E   E   NL4-3 genbank.SEQ
1782 AAG ACT ATT TTA AAA GCA TTG GGA CCA GGA GCG ACA CTA GAA GAA
2309  K   T   I   L   K   A   L   G   P   G   A   T   L   E   E   pHDMHgpm2.seq
2309 AAG ACC ATC CTG AAG GCC CTG GGC CCC GGC GCC ACC CTG GAG GAG

        1830                                      1860
1827  M   M   T   A   C   Q   G   V   G   G   P   G   H   K   A   NL4-3 genbank.SEQ
1827 ATG ATG ACA GCA TGT CAG GGA GTG GGG GGA CCC GGC CAT AAA GCA
2354  M   M   T   A   C   Q   G   V   G   G   P   G   H   K   A   pHDMHgpm2.seq
2354 ATG ATG ACC GCC TGC CAG GGC GTG GGC GGC CCC GGC CAC AAG GCC
```

Fig. 8C

Alignment Report of Codon optimization (gag).MEG, using Clustal method with PAM250 residue weight table.

```
                                        1890
1872  R   V   L   A   E   A   M   S   Q   V   T   N   P   A   T    NL4-3 genbank.SEQ
1872 AGA GTT TTG GCT GAA GCA ATG AGC CAA GTA ACA AAT CCA GCT ACC
2399  R   V   L   A   E   A   M   S   Q   V   T   N   P   A   T    pHDMHgpm2.seq
2399 CGC GTG CTG GCC GAG GCC ATG TCC CAA GTC ACC AAC CCC GCC ACC

        1920                                        1950
1917  I   M   I   Q   K   G   N   F   R   N   Q   R   K   T   V    NL4-3 genbank.SEQ
1917 ATA ATG ATA CAG AAA GGC AAT TTT AGG AAC CAA AGA AAG ACT GTT
2444  I   M   I   Q   K   G   N   F   R   N   Q   R   K   T   V    pHDMHgpm2.seq
2444 ATC ATG ATC CAG AAG GGC AAC TTC CGC AAC CAG CGC AAG ACC GTG

                                        1980
1962  K   C   F   N   C   G   K   E   G   H   I   A   K   N   C    NL4-3 genbank.SEQ
1962 AAG TGT TTC AAT TGT GGC AAA GAA GGG CAC ATA GCC AAA AAT TGC
2489  K   C   F   N   C   G   K   E   G   H   I   A   K   N   C    pHDMHgpm2.seq
2489 AAG TGC TTC AAC TGC GGC AAG GAG GGC CAC ATC GCC AAG AAC TGC

        2010                                        2040
2007  R   A   P   R   K   K   G   C   W   K   C   G   K   E   G    NL4-3 genbank.SEQ
2007 AGG GCC CCT AGG AAA AAG GGC TGT TGG AAA TGT GGA AAG GAA GGA
2534  R   A   P   R   K   K   G   C   W   K   C   G   K   E   G    pHDMHgpm2.seq
2534 CGC GCC CCC CGC AAG AAG GGC TGC TGG AAG TGC GGC AAG GAG GGC

                                        2070
2052  H   Q   M   K   D   C   T   E   R   Q   A   N   F   L   G    NL4-3 genbank.SEQ
2052 CAC CAA ATG AAA GAT TGT ACT GAG AGA CAG GCT AAT TTT TTA GGG
2579  H   Q   M   K   D   C   T   E   R   Q   A   N   F   L   G    pHDMHgpm2.seq
2579 CAC CAG ATG AAA GAT TGT ACT GAG AGA CAG GCT AAT TTT TTA GGG

        2100                                        2130
2097  K   I   W   P   S   H   K   G   R   P   G   N   F   L   Q    NL4-3 genbank.SEQ
2097 AAG ATC TGG CCT TCC CAC AAG GGA AGG CCA GGG AAT TTT CTT CAG
2624  K   I   W   P   S   H   K   G   R   P   G   N   F   L   Q    pHDMHgpm2.seq
2624 AAG ATC TGG CCT TCC CAC AAG GGA AGG CCA GGG AAT TTT CTT CAG

                                        2160
2142  S   R   P   E   P   T   A   P   P   E   E   S   F   R   F    NL4-3 genbank.SEQ
2142 AGC AGA CCA GAG CCA ACA GCC CCA CCA GAA GAG AGC TTC AGG TTT
2669  S   R   P   E   P   T   A   P   P   E   E   S   F   R   F    pHDMHgpm2.seq
2669 AGC AGA CCA GAG CCA ACA GCC CCA CCA GAA GAG AGC TTC AGG TTT

        2190                                        2220
2187  G   E   E   T   T   T   P   S   Q   K   Q   E   P   I   D    NL4-3 genbank.SEQ
2187 GGG GAA GAG ACA ACA ACT CCC TCT CAG AAG CAG GAG CCG ATA GAC
2714  G   E   E   T   T   T   P   S   Q   K   Q   E   P   I   D    pHDMHgpm2.seq
2714 GGG GAA GAG ACA ACA ACT CCC TCT CAG AAG CAG GAG CCG ATA GAC
```

Fig. 8D

Alignment Report of Codon optimization (gag).MEG, using Clustal method with PAM250 residue weight table.

```
                                     2250
2232  K   E   L   Y   P   L   A   S   L   R   S   L   F   G   S    NL4-3 genbank.SEQ
2232  AAG GAA CTG TAT CCT TTA GCT TCC CTC AGA TCA CTC TTT GGC AGC
2759  K   E   L   Y   P   L   A   S   L   R   S   L   F   G   S    pHDMHgpm2.seq
2759  AAG GAA CTG TAT CCT TTA GCT TCC CTC AGA TCA CTC TTT GGC AGC

         2280
2277  D   P   S   S   Q                                            NL4-3 genbank.SEQ
2277  GAC CCC TCG TCA CAA TAA
2804  D   P   S   S   Q                                            pHDMHgpm2.seq
2804  GAC CCC TCG TCA CAA TAA
```

Fig. 8E

Alignment Report of Codon Optimization (pol).MEG, using Clustal method with PAM250 residue weight table.

```
       2090                                                   2120
2087   F   F   R   E   D   L   A   F   P   Q   G   K   A   R   E    NL4-3 genbank.SEQ
2087  TTT TTT AGG GAA GAT CTG GCC TTC CCA CAA GGG AAG GCC AGG GAA
2085   F   F   R   E   D   L   A   F   P   Q   G   K   A   R   E    pNL4-3.seq
2085  TTT TTT AGG GAA GAT CTG GCC TTC CCA CAA GGG AAG GCC AGG GAA
2612   F   F   R   E   D   L   A   F   P   Q   G   K   A   R   E    pHDMHgpm2.seq
2612  TTT TTT AGG GAA GAT CTG GCC TTC CCA CAA GGG AAG GCC AGG GAA

                                2150
2132   F   S   S   E   Q   T   R   A   N   S   P   T   R   R   E    NL4-3 genbank.SEQ
2132  TTT TCT TCA GAG CAG ACC AGA GCC AAC AGC CCC ACC AGA AGA GAG
2130   F   S   S   E   Q   T   R   A   N   S   P   T   R   R   E    pNL4-3.seq
2130  TTT TCT TCA GAG CAG ACC AGA GCC AAC AGC CCC ACC AGA AGA GAG
2657   F   S   S   E   Q   T   R   A   N   S   P   T   R   R   E    pHDMHgpm2.seq
2657  TTT TCT TCA GAG CAG ACC AGA GCC AAC AGC CCC ACC AGA AGA GAG

       2180                                                   2210
2177   L   Q   V   W   G   R   D   N   N   S   L   S   E   A   G    NL4-3 genbank.SEQ
2177  CTT CAG GTT TGG GGA AGA GAC AAC AAC TCC CTC TCA GAA GCA GGA
2175   L   Q   V   W   G   R   D   N   N   S   L   S   E   A   G    pNL4-3.seq
2175  CTT CAG GTT TGG GGA AGA GAC AAC AAC TCC CTC TCA GAA GCA GGA
2702   L   Q   V   W   G   R   D   N   N   S   L   S   E   A   G    pHDMHgpm2.seq
2702  CTT CAG GTT TGG GGA AGA GAC AAC AAC TCC CTC TCA GAA GCA GGA

                                2240
2222   A   D   R   Q   G   T   V   S   F   S   F   P   Q   I   T    NL4-3 genbank.SEQ
2222  GCC GAT AGA CAA GGA ACT GTA TCC TTT AGC TTC CCT CAG ATC ACT
2220   A   D   R   Q   G   T   V   S   F   S   F   P   Q   I   T    pNL4-3.seq
2220  GCC GAT AGA CAA GGA ACT GTA TCC TTT AGC TTC CCT CAG ATC ACT
2747   A   D   R   Q   G   T   V   S   F   S   F   P   Q   I   T    pHDMHgpm2.seq
2747  GCC GAT AGA CAA GGA ACT GTA TCC TTT AGC TTC CCT CAG ATC ACT

       2270                                                   2300
2267   L   W   Q   R   P   L   V   T   I   K   I   G   G   Q   L    NL4-3 genbank.SEQ
2267  CTT TGG CAG CGA CCC CTC GTC ACA ATA AAG ATA GGG GGG CAA TTA
2265   L   W   Q   R   P   L   V   T   I   K   I   G   G   Q   L    pNL4-3.seq
2265  CTT TGG CAG CGA CCC CTC GTC ACA ATA AAG ATA GGG GGG CAA TTA
2792   L   W   Q   R   P   L   V   T   I   K   I   G   G   Q   L    pHDMHgpm2.seq
2792  CTT TGG CAG CGA CCC CTC GTC ACA ATA AAG ATC GGT GGC CAG CTG

                                2330
2312   K   E   A   L   L   D   T   G   A   D   D   T   V   L   E    NL4-3 genbank.SEQ
2312  AAG GAA GCT CTA TTA GAT ACA GGA GCA GAT GAT ACA GTA TTA GAA
2310   K   E   A   L   L   D   T   G   A   D   D   T   V   L   E    pNL4-3.seq
2310  AAG GAA GCT CTA TTA GAT ACA GGA GCA GAT GAT ACA GTA TTA GAA
2837   K   E   A   L   L   D   T   G   A   D   D   T   V   L   E    pHDMHgpm2.seq
2837  AAG GAG GCC CTG CTG GAC ACC GGC GCC GAC GAC ACC GTG CTG GAG
```

Fig. 9A

Alignment Report of Codon Optimization (pol).MEG, using Clustal method with PAM250 residue weight table.

```
        2360                                    2390
2357  E   M   N   L   P   G   R   W   K   P   K   M   I   G   G    NL4-3 genbank.SEQ
2357 GAA ATG AAT TTG CCA GGA AGA TGG AAA CCA AAA ATG ATA GGG GGA
2355  E   M   N   L   P   G   R   W   K   P   K   M   I   G   G    pNL4-3.seq
2355 GAA ATG AAT TTG CCA GGA AGA TGG AAA CCA AAA ATG ATA GGG GGA
2882  E   M   N   L   P   G   R   W   K   P   K   M   I   G   G    pHDMHgpm2.seq
2882 GAG ATG AAC CTG CCC GGC CGC TGG AAG CCC AAG ATG ATC GGC GGC

                             2420
2402  I   G   G   F   I   K   V   G   Q   Y   D   Q   I   L   I    NL4-3 genbank.SEQ
2402 ATT GGA GGT TTT ATC AAA GTA GGA CAG TAT GAT CAG ATA CTC ATA
2400  I   G   G   F   I   K   V   R   Q   Y   D   Q   I   L   I    pNL4-3.seq
2400 ATT GGA GGT TTT ATC AAA GTA AGA CAG TAT GAT CAG ATA CTC ATA
2927  I   G   G   F   I   K   V   R   Q   Y   D   Q   I   L   I    pHDMHgpm2.seq
2927 ATC GGC GGC TTC ATC AAA GTC CGC CAG TAC GAC CAG ATC CTG ATC

        2450                                    2480
2447  E   I   C   G   H   K   A   I   G   T   V   L   V   G   P    NL4-3 genbank.SEQ
2447 GAA ATC TGC GGA CAT AAA GCT ATA GGT ACA GTA TTA GTA GGA CCT
2445  E   I   C   G   H   K   A   I   G   T   V   L   V   G   P    pNL4-3.seq
2445 GAA ATC TGC GGA CAT AAA GCT ATA GGT ACA GTA TTA GTA GGA CCT
2972  E   I   C   G   H   K   A   I   G   T   V   L   V   G   P    pHDMHgpm2.seq
2972 GAG ATC TGC GGC CAC AAG GCC ATC GGC ACC GTG CTG GTG GGC CCC

                             2510
2492  T   P   V   N   I   I   G   R   N   L   L   T   Q   I   G    NL4-3 genbank.SEQ
2492 ACA CCT GTC AAC ATA ATT GGA AGA AAT CTG TTG ACT CAG ATT GGC
2490  T   P   V   N   I   I   G   R   N   L   L   T   Q   I   G    pNL4-3.seq
2490 ACA CCT GTC AAC ATA ATT GGA AGA AAT CTG TTG ACT CAG ATT GGC
3017  T   P   V   N   I   I   G   R   N   L   L   T   Q   I   G    pHDMHgpm2.seq
3017 ACC CCC GTG AAC ATC ATC GGC CGC AAC CTG CTG ACC CAG ATC GGC

        2540                                    2570
2537  C   T   L   N   F   P   I   S   P   I   E   T   V   P   V    NL4-3 genbank.SEQ
2537 TGC ACT TTA AAT TTT CCC ATT AGT CCT ATT GAG ACT GTA CCA GTA
2535  C   T   L   N   F   P   I   S   P   I   E   T   V   P   V    pNL4-3.seq
2535 TGC ACT TTA AAT TTT CCC ATT AGT CCT ATT GAG ACT GTA CCA GTA
3062  C   T   L   N   F   P   I   S   P   I   E   T   V   P   V    pHDMHgpm2.seq
3062 TGC ACC CTG AAC TTC CCC ATC TCC CCC ATC GAG ACC GTG CCC GTG

                             2600
2582  K   L   K   P   G   M   D   G   P   K   V   K   Q   W   P    NL4-3 genbank.SEQ
2582 AAA TTA AAG CCA GGA ATG GAT GGC CCA AAA GTT AAA CAA TGG CCA
2580  K   L   K   P   G   M   D   G   P   K   V   K   Q   W   P    pNL4-3.seq
2580 AAA TTA AAG CCA GGA ATG GAT GGC CCA AAA GTT AAA CAA TGG CCA
3107  K   L   K   P   G   M   D   G   P   K   V   K   Q   W   P    pHDMHgpm2.seq
3107 AAG CTG AAG CCC GGC ATG GAC GGC CCC AAA GTC AAG CAG TGG CCC
```

Fig. 9B

Alignment Report of Codon Optimization (pol).MEG, using Clustal method with PAM250 residue weight table.

```
          2630                                       2660
2627  L   T   E   E   K   I   K   A   L   V   E   I   C   T   E    NL4-3 genbank.SEQ
2627 TTG ACA GAA GAA AAA ATA AAA GCA TTA GTA GAA ATT TGT ACA GAA
2625  L   T   E   E   K   I   K   A   L   V   E   I   C   T   E    pNL4-3.seq
2625 TTG ACA GAA GAA AAA ATA AAA GCA TTA GTA GAA ATT TGT ACA GAA
3152  L   T   E   E   K   I   K   A   L   V   E   I   C   T   E    pHDMHgpm2.seq
3152 CTG ACC GAG GAG AAG ATC AAG GCC CTG GTG GAG ATC TGC ACC GAG

                             2690
2672  M   E   K   E   G   K   I   S   K   I   G   P   E   N   P    NL4-3 genbank.SEQ
2672 ATG GAA AAG GAA GGA AAA ATT TCA AAA ATT GGG CCT GAA AAT CCA
2670  M   E   K   E   G   K   I   S   K   I   G   P   E   N   P    pNL4-3.seq
2670 ATG GAA AAG GAA GGA AAA ATT TCA AAA ATT GGG CCT GAA AAT CCA
3197  M   E   K   E   G   K   I   S   K   I   G   P   E   N   P    pHDMHgpm2.seq
3197 ATG GAG AAG GAG GGC AAG ATC TCC AAG ATC GGC CCC GAG AAC CCC

          2720                                       2750
2717  Y   N   T   P   V   F   A   I   K   K   K   D   S   T   K    NL4-3 genbank.SEQ
2717 TAC AAT ACT CCA GTA TTT GCC ATA AAG AAA AAA GAC AGT ACT AAA
2715  Y   N   T   P   V   F   A   I   K   K   K   D   S   T   K    pNL4-3.seq
2715 TAC AAT ACT CCA GTA TTT GCC ATA AAG AAA AAA GAC AGT ACT AAA
3242  Y   N   T   P   V   F   A   I   K   K   K   D   S   T   K    pHDMHgpm2.seq
3242 TAC AAC ACC CCC GTG TTC GCC ATC AAG AAG AAG GAC TCC ACC AAG

                             2780
2762  W   R   K   L   V   D   F   R   E   L   N   K   R   T   Q    NL4-3 genbank.SEQ
2762 TGG AGA AAA TTA GTA GAT TTC AGA GAA CTT AAT AAG AGA ACT CAA
2760  W   R   K   L   V   D   F   R   E   L   N   K   R   T   Q    pNL4-3.seq
2760 TGG AGA AAA TTA GTA GAT TTC AGA GAA CTT AAT AAG AGA ACT CAA
3287  W   R   K   L   V   D   F   R   E   L   N   K   R   T   Q    pHDMHgpm2.seq
3287 TGG CGC AAG CTG GTG GAC TTC CGC GAG CTG AAC AAG CGC ACC CAG

          2810                                       2840
2807  D   F   W   E   V   Q   L   G   I   P   H   P   A   G   L    NL4-3 genbank.SEQ
2807 GAT TTC TGG GAA GTT CAA TTA GGA ATA CCA CAT CCT GCA GGG TTA
2805  D   F   W   E   V   Q   L   G   I   P   H   P   A   G   L    pNL4-3.seq
2805 GAT TTC TGG GAA GTT CAA TTA GGA ATA CCA CAT CCT GCA GGG TTA
3332  D   F   W   E   V   Q   L   G   I   P   H   P   A   G   L    pHDMHgpm2.seq
3332 GAC TTC TGG GAG GTG CAG CTG GGC ATC CCC CAC CCC GCC GGC CTG

                             2870
2852  K   Q   K   K   S   V   T   V   L   D   V   G   D   A   Y    NL4-3 genbank.SEQ
2852 AAA CAG AAA AAA TCA GTA ACA GTA CTG GAT GTG GGC GAT GCA TAT
2850  K   Q   K   K   S   V   T   V   L   D   V   G   D   A   Y    pNL4-3.seq
2850 AAA CAG AAA AAA TCA GTA ACA GTA CTG GAT GTG GGC GAT GCA TAT
3377  K   Q   K   K   S   V   T   V   L   D   V   G   D   A   Y    pHDMHgpm2.seq
3377 AAG CAG AAG AAG TCC GTG ACC GTG CTG GAC GTG GGC GAC GCC TAC
```

Fig. 9C

Alignment Report of Codon Optimization (pol).MEG, using Clustal method with PAM250 residue weight table.

2900 2930

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2897 | F | S | V | P | L | D | K | D | F | R | K | Y | T | A | F | NL4-3 genbank.SEQ |
| 2897 | TTT | TCA | GTT | CCC | TTA | GAT | AAA | GAC | TTC | AGG | AAG | TAT | ACT | GCA | TTT | |
| 2895 | F | S | V | P | L | D | K | D | F | R | K | Y | T | A | F | pNL4-3.seq |
| 2895 | TTT | TCA | GTT | CCC | TTA | GAT | AAA | GAC | TTC | AGG | AAG | TAT | ACT | GCA | TTT | |
| 3422 | F | S | V | P | L | D | K | D | F | R | K | Y | T | A | F | pHDMHgpm2.seq |
| 3422 | TTC | TCC | GTG | CCC | CTG | GAC | AAG | GAC | TTC | CGC | AAG | TAC | ACC | GCC | TTC | |

2960

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2942 | T | I | P | S | I | N | N | E | T | P | G | I | R | Y | Q | NL4-3 genbank.SEQ |
| 2942 | ACC | ATA | CCT | AGT | ATA | AAC | AAT | GAG | ACA | CCA | GGG | ATT | AGA | TAT | CAG | |
| 2940 | T | I | P | S | I | N | N | E | T | P | G | I | R | Y | Q | pNL4-3.seq |
| 2940 | ACC | ATA | CCT | AGT | ATA | AAC | AAT | GAG | ACA | CCA | GGG | ATT | AGA | TAT | CAG | |
| 3467 | T | I | P | S | I | N | N | E | T | P | G | I | R | Y | Q | pHDMHgpm2.seq |
| 3467 | ACC | ATC | CCC | TCC | ATC | AAC | AAC | GAG | ACC | CCC | GGC | ATC | CGC | TAC | CAG | |

2990 3020

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2987 | Y | N | V | L | P | Q | G | W | K | G | S | P | A | I | F | NL4-3 genbank.SEQ |
| 2987 | TAC | AAT | GTG | CTT | CCA | CAG | GGA | TGG | AAA | GGA | TCA | CCA | GCA | ATA | TTC | |
| 2985 | Y | N | V | L | P | Q | G | W | K | G | S | P | A | I | F | pNL4-3.seq |
| 2985 | TAC | AAT | GTG | CTT | CCA | CAG | GGA | TGG | AAA | GGA | TCA | CCA | GCA | ATA | TTC | |
| 3512 | Y | N | V | L | P | Q | G | W | K | G | S | P | A | I | F | pHDMHgpm2.seq |
| 3512 | TAC | AAC | GTG | CTG | CCC | CAG | GGC | TGG | AAG | GGC | TCC | CCC | GCC | ATC | TTC | |

3050

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3032 | Q | C | S | M | T | K | I | L | E | P | F | R | K | Q | N | NL4-3 genbank.SEQ |
| 3032 | CAG | TGT | AGC | ATG | ACA | AAA | ATC | TTA | GAG | CCT | TTT | AGA | AAA | CAA | AAT | |
| 3030 | Q | C | S | M | T | K | I | L | E | P | F | R | K | Q | N | pNL4-3.seq |
| 3030 | CAG | TGT | AGC | ATG | ACA | AAA | ATC | TTA | GAG | CCT | TTT | AGA | AAA | CAA | AAT | |
| 3557 | Q | C | S | M | T | K | I | L | E | P | F | R | K | Q | N | pHDMHgpm2.seq |
| 3557 | CAG | TGC | TCC | ATG | ACC | AAG | ATC | CTG | GAG | CCC | TTC | CGC | AAG | CAG | AAC | |

3080 3110

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3077 | P | D | I | V | I | Y | Q | Y | M | D | D | L | Y | V | G | NL4-3 genbank.SEQ |
| 3077 | CCA | GAC | ATA | GTC | ATC | TAT | CAA | TAC | ATG | GAT | GAT | TTG | TAT | GTA | GGA | |
| 3075 | P | D | I | V | I | Y | Q | Y | M | D | D | L | Y | V | G | pNL4-3.seq |
| 3075 | CCA | GAC | ATA | GTC | ATC | TAT | CAA | TAC | ATG | GAT | GAT | TTG | TAT | GTA | GGA | |
| 3602 | P | D | I | V | I | Y | Q | Y | M | D | D | L | Y | V | G | pHDMHgpm2.seq |
| 3602 | CCC | GAC | ATC | GTG | ATC | TAC | CAG | TAC | ATG | GAC | GAC | CTG | TAC | GTG | GGC | |

3140

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3122 | S | D | L | E | I | G | Q | H | R | T | K | I | E | E | L | NL4-3 genbank.SEQ |
| 3122 | TCT | GAC | TTA | GAA | ATA | GGG | CAG | CAT | AGA | ACA | AAA | ATA | GAG | GAA | CTG | |
| 3120 | S | D | L | E | I | G | Q | H | R | T | K | I | E | E | L | pNL4-3.seq |
| 3120 | TCT | GAC | TTA | GAA | ATA | GGG | CAG | CAT | AGA | ACA | AAA | ATA | GAG | GAA | CTG | |
| 3647 | S | D | L | E | I | G | Q | H | R | T | K | I | E | E | L | pHDMHgpm2.seq |
| 3647 | TCC | GAC | CTG | GAG | ATC | GGC | CAG | CAC | CGC | ACC | AAG | ATC | GAG | GAG | CTG | |

Fig. 9D

Alignment Report of Codon Optimization (pol).MEG, using Clustal method with PAM250 residue weight table.

| | 3170 | | | | | | | | | | 3200 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3167 | R | Q | H | L | L | R | W | G | F | T | T | P | D | K | K NL4-3 genbank.SEQ |
| 3167 | AGA | CAA | CAT | CTG | TTG | AGG | TGG | GGA | TTT | ACC | ACA | CCA | GAC | AAA | AAA |
| 3165 | R | Q | H | L | L | R | W | G | F | T | T | P | D | K | K pNL4-3.seq |
| 3165 | AGA | CAA | CAT | CTG | TTG | AGG | TGG | GGA | TTT | ACC | ACA | CCA | GAC | AAA | AAA |
| 3692 | R | Q | H | L | L | R | W | G | F | T | T | P | D | K | K pHDMHgpm2.seq |
| 3692 | CGC | CAG | CAC | CTG | CTG | CGC | TGG | GGC | TTC | ACC | ACC | CCC | GAC | AAG | AAG |

| | | | | | | | 3230 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3212 | H | Q | K | E | P | P | F | L | W | M | G | Y | E | L | H NL4-3 genbank.SEQ |
| 3212 | CAT | CAG | AAA | GAA | CCT | CCA | TTC | CTT | TGG | ATG | GGT | TAT | GAA | CTC | CAT |
| 3210 | H | Q | K | E | P | P | F | L | W | M | G | Y | E | L | H pNL4-3.seq |
| 3210 | CAT | CAG | AAA | GAA | CCT | CCA | TTC | CTT | TGG | ATG | GGT | TAT | GAA | CTC | CAT |
| 3737 | H | Q | K | E | P | P | F | L | W | M | G | Y | E | L | H pHDMHgpm2.seq |
| 3737 | CAC | CAG | AAG | GAG | CCC | CCC | TTC | CTG | TGG | ATG | GGC | TAC | GAG | CTG | CAC |

| | 3260 | | | | | | | | | | 3290 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3257 | P | D | K | W | T | V | Q | P | I | V | L | P | E | K | D NL4-3 genbank.SEQ |
| 3257 | CCT | GAT | AAA | TGG | ACA | GTA | CAG | CCT | ATA | GTG | CTG | CCA | GAA | AAG | GAC |
| 3255 | P | D | K | W | T | V | Q | P | I | V | L | P | E | K | D pNL4-3.seq |
| 3255 | CCT | GAT | AAA | TGG | ACA | GTA | CAG | CCT | ATA | GTG | CTG | CCA | GAA | AAG | GAC |
| 3782 | P | D | K | W | T | V | Q | P | I | V | L | P | E | K | D pHDMHgpm2.seq |
| 3782 | CCC | GAC | AAG | TGG | ACC | GTG | CAG | CCC | ATC | GTG | CTG | CCC | GAG | AAG | GAC |

| | | | | | | | 3320 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3302 | S | W | T | V | N | D | I | Q | K | L | V | G | K | L | N NL4-3 genbank.SEQ |
| 3302 | AGC | TGG | ACT | GTC | AAT | GAC | ATA | CAG | AAA | TTA | GTG | GGA | AAA | TTG | AAT |
| 3300 | S | W | T | V | N | D | I | Q | K | L | V | G | K | L | N pNL4-3.seq |
| 3300 | AGC | TGG | ACT | GTC | AAT | GAC | ATA | CAG | AAA | TTA | GTG | GGA | AAA | TTG | AAT |
| 3827 | S | W | T | V | N | D | I | Q | K | L | V | G | K | L | N pHDMHgpm2.seq |
| 3827 | TCC | TGG | ACC | GTG | AAC | GAC | ATC | CAG | AAG | CTG | GTG | GGC | AAG | CTG | AAC |

| | 3350 | | | | | | | | | | 3380 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3347 | W | A | S | Q | I | Y | A | G | I | K | V | R | Q | L | C NL4-3 genbank.SEQ |
| 3347 | TGG | GCA | AGT | CAG | ATT | TAT | GCA | GGG | ATT | AAA | GTA | AGG | CAA | TTA | TGT |
| 3345 | W | A | S | Q | I | Y | A | G | I | K | V | R | Q | L | C pNL4-3.seq |
| 3345 | TGG | GCA | AGT | CAG | ATT | TAT | GCA | GGG | ATT | AAA | GTA | AGG | CAA | TTA | TGT |
| 3872 | W | A | S | Q | I | Y | A | G | I | K | V | R | Q | L | C pHDMHgpm2.seq |
| 3872 | TGG | GCC | TCC | CAG | ATC | TAC | GCC | GGC | ATC | AAA | GTC | CGC | CAG | CTG | TGC |

| | | | | | | | 3410 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3392 | K | L | L | R | G | T | K | A | L | T | E | V | V | P | L NL4-3 genbank.SEQ |
| 3392 | AAA | CTT | CTT | AGG | GGA | ACC | AAA | GCA | CTA | ACA | GAA | GTA | GTA | CCA | CTA |
| 3390 | K | L | L | R | G | T | K | A | L | T | E | V | V | P | L pNL4-3.seq |
| 3390 | AAA | CTT | CTT | AGG | GGA | ACC | AAA | GCA | CTA | ACA | GAA | GTA | GTA | CCA | CTA |
| 3917 | K | L | L | R | G | T | K | A | L | T | E | V | V | P | L pHDMHgpm2.seq |
| 3917 | AAG | CTG | CTG | CGC | GGC | ACC | AAG | GCC | CTG | ACC | GAG | GTG | GTG | CCC | CTG |

Fig. 9E

Alignment Report of Codon Optimization (pol).MEG, using Clustal method with PAM250 residue weight table.

```
         3440                                              3470
3437  T   E   E   A   E   L   E   L   A   E   N   R   E   I   L   NL4-3 genbank.SEQ
3437 ACA GAA GAA GCA GAG CTA GAA CTG GCA GAA AAC AGG GAG ATT CTA
3435  T   E   E   A   E   L   E   L   A   E   N   R   E   I   L   pNL4-3.seq
3435 ACA GAA GAA GCA GAG CTA GAA CTG GCA GAA AAC AGG GAG ATT CTA
3962  T   E   E   A   E   L   E   L   A   E   N   R   E   I   L   pHDMHgpm2.seq
3962 ACC GAG GAG GCC GAG CTG GAG CTG GCC GAG AAC CGC GAG ATC CTG

                                  3500
3482  K   E   P   V   H   G   V   Y   Y   D   P   S   K   D   L   NL4-3 genbank.SEQ
3482 AAA GAA CCG GTA CAT GGA GTG TAT TAT GAC CCA TCA AAA GAC TTA
3480  K   E   P   V   H   G   V   Y   Y   D   P   S   K   D   L   pNL4-3.seq
3480 AAA GAA CCG GTA CAT GGA GTG TAT TAT GAC CCA TCA AAA GAC TTA
4007  K   E   P   V   H   G   V   Y   Y   D   P   S   K   D   L   pHDMHgpm2.seq
4007 AAG GAG CCC GTG CAC GGC GTG TAC TAC GAC CCC TCC AAG GAC CTG

         3530                                              3560
3527  I   A   E   I   Q   K   Q   G   Q   G   Q   W   T   Y   Q   NL4-3 genbank.SEQ
3527 ATA GCA GAA ATA CAG AAG CAG GGG CAA GGC CAA TGG ACA TAT CAA
3525  I   A   E   I   Q   K   Q   G   Q   G   Q   W   T   Y   Q   pNL4-3.seq
3525 ATA GCA GAA ATA CAG AAG CAG GGG CAA GGC CAA TGG ACA TAT CAA
4052  I   A   E   I   Q   K   Q   G   Q   G   Q   W   T   Y   Q   pHDMHgpm2.seq
4052 ATC GCC GAG ATC CAG AAG CAG GGC CAG GGC CAG TGG ACC TAC CAG

                                  3590
3572  I   Y   Q   E   P   F   K   N   L   K   T   G   K   Y   A   NL4-3 genbank.SEQ
3572 ATT TAT CAA GAG CCA TTT AAA AAT CTG AAA ACA GGA AAA TAT GCA
3570  I   Y   Q   E   P   F   K   N   L   K   T   G   K   Y   A   pNL4-3.seq
3570 ATT TAT CAA GAG CCA TTT AAA AAT CTG AAA ACA GGA AAA TAT GCA
4097  I   Y   Q   E   P   F   K   N   L   K   T   G   K   Y   A   pHDMHgpm2.seq
4097 ATC TAC CAG GAG CCC TTC AAG AAC CTG AAG ACC GGC AAA TAC GCC

         3620                                              3650
3617  R   M   K   G   A   H   T   N   D   V   K   Q   L   T   E   NL4-3 genbank.SEQ
3617 AGA ATG AAG GGT GCC CAC ACT AAT GAT GTG AAA CAA TTA ACA GAG
3615  R   M   K   G   A   H   T   N   D   V   K   Q   L   T   E   pNL4-3.seq
3615 AGA ATG AAG GGT GCC CAC ACT AAT GAT GTG AAA CAA TTA ACA GAG
4142  R   M   K   G   A   H   T   N   D   V   K   Q   L   T   E   pHDMHgpm2.seq
4142 CGC ATG AAG GGC GCC CAC ACC AAC GAC GTG AAG CAG CTG ACC GAG

                                  3680
3662  A   V   Q   K   I   A   T   E   S   I   V   I   W   G   K   NL4-3 genbank.SEQ
3662 GCA GTA CAA AAA ATA GCC ACA GAA AGC ATA GTA ATA TGG GGA AAG
3660  A   V   Q   K   I   A   T   E   S   I   V   I   W   G   K   pNL4-3.seq
3660 GCA GTA CAA AAA ATA GCC ACA GAA AGC ATA GTA ATA TGG GGA AAG
4187  A   V   Q   K   I   A   T   E   S   I   V   I   W   G   K   pHDMHgpm2.seq
4187 GCC GTG CAG AAG ATC GCC ACC GAG TCC ATC GTG ATC TGG GGC AAG
```

Fig. 9F

Alignment Report of Codon Optimization (pol).MEG, using Clustal method with PAM250 residue weight table.

```
           3710                                              3740
3707  T   P   K   F   K   L   P   I   Q   K   E   T   W   E   A   NL4-3 genbank.SEQ
3707 ACT CCT AAA TTT AAA TTA CCC ATA CAA AAG GAA ACA TGG GAA GCA
3705  T   P   K   F   K   L   P   I   Q   K   E   T   W   E   A   pNL4-3.seq
3705 ACT CCT AAA TTT AAA TTA CCC ATA CAA AAG GAA ACA TGG GAA GCA
4232  T   P   K   F   K   L   P   I   Q   K   E   T   W   E   A   pHDMHgpm2.seq
4232 ACT CCC AAG TTC AAG CTG CCC ATC CAG AAG GAG ACC TGG GAG GCC

                                3770
3752  W   W   T   E   Y   W   Q   A   T   W   I   P   E   W   E   NL4-3 genbank.SEQ
3752 TGG TGG ACA GAG TAT TGG CAA GCC ACC TGG ATT CCT GAG TGG GAG
3750  W   W   T   E   Y   W   Q   A   T   W   I   P   E   W   E   pNL4-3.seq
3750 TGG TGG ACA GAG TAT TGG CAA GCC ACC TGG ATT CCT GAG TGG GAG
4277  W   W   T   E   Y   W   Q   A   T   W   I   P   E   W   E   pHDMHgpm2.seq
4277 TGG TGG ACC GAG TAC TGG CAG GCC ACC TGG ATC CCC GAG TGG GAG

        3800                                              3830
3797  F   V   N   T   P   P   L   V   K   L   W   Y   Q   L   E   NL4-3 genbank.SEQ
3797 TTT GTC AAT ACC CCT CCC TTA GTG AAG TTA TGG TAC CAG TTA GAG
3795  F   V   N   T   P   P   L   V   K   L   W   Y   Q   L   E   pNL4-3.seq
3795 TTT GTC AAT ACC CCT CCC TTA GTG AAG TTA TGG TAC CAG TTA GAG
4322  F   V   N   T   P   P   L   V   K   L   W   Y   Q   L   E   pHDMHgpm2.seq
4322 TTC GTG AAC ACC CCC CCC CTG GTG AAG CTG TGG TAC CAG CTG GAG

                                3860
3842  K   E   P   I   I   G   A   E   T   F   Y   V   D   G   A   NL4-3 genbank.SEQ
3842 AAA GAA CCC ATA ATA GGA GCA GAA ACT TTC TAT GTA GAT GGG GCA
3840  K   E   P   I   I   G   A   E   T   F   Y   V   D   G   A   pNL4-3.seq
3840 AAA GAA CCC ATA ATA GGA GCA GAA ACT TTC TAT GTA GAT GGG GCA
4367  K   E   P   I   I   G   A   E   T   F   Y   V   D   G   A   pHDMHgpm2.seq
4367 AAG GAG CCC ATC ATC GGC GCC GAG ACC TTC TAC GTG GAC GGC GCC

        3890                                              3920
3887  A   N   R   E   T   K   L   G   K   A   G   Y   V   T   D   NL4-3 genbank.SEQ
3887 GCC AAT AGG GAA ACT AAA TTA GGA AAA GCA GGA TAT GTA ACT GAC
3885  A   N   R   E   T   K   L   G   K   A   G   Y   V   T   D   pNL4-3.seq
3885 GCC AAT AGG GAA ACT AAA TTA GGA AAA GCA GGA TAT GTA ACT GAC
4412  A   N   R   E   T   K   L   G   K   A   G   Y   V   T   D   pHDMHgpm2.seq
4412 GCC AAC CGC GAG ACC AAG CTG GGC AAG GCC GGC TAC GTG ACC GAC

                                3950
3932  R   G   R   Q   K   V   V   P   L   T   D   T   T   N   Q   NL4-3 genbank.SEQ
3932 AGA GGA AGA CAA AAA GTT GTC CCC CTA ACG GAC ACA ACA AAT CAG
3930  R   G   R   Q   K   V   V   P   L   T   D   T   T   N   Q   pNL4-3.seq
3930 AGA GGA AGA CAA AAA GTT GTC CCC CTA ACG GAC ACA ACA AAT CAG
4457  R   G   R   Q   K   V   V   P   L   T   D   T   T   N   Q   pHDMHgpm2.seq
4457 CGC GGC CGC CAG AAG GTG GTG CCC CTG ACC GAC ACC ACC AAC CAG
```

Fig. 9G

Alignment Report of Codon Optimization (pol).MEG, using Clustal method with PAM250 residue weight table.

```
           3980                                              4010
3977   K   T   E   L   Q   A   I   H   L   A   L   Q   D   S   G   NL4-3 genbank.SEQ
3977  AAG ACT GAG TTA CAA GCA ATT CAT CTA GCT TTG CAG GAT TCG GGA
3975   K   T   E   L   Q   A   I   H   L   A   L   Q   D   S   G   pNL4-3.seq
3975  AAG ACT GAG TTA CAA GCA ATT CAT CTA GCT TTG CAG GAT TCG GGA
4502   K   T   E   L   Q   A   I   H   L   A   L   Q   D   S   G   pHDMHgpm2.seq
4502  AAG ACC GAG CTG CAG GCC ATC CAC CTG GCC CTG CAA GAC TCC GGC

                                 4040
4022   L   E   V   N   I   V   T   D   S   Q   Y   A   L   G   I   NL4-3 genbank.SEQ
4022  TTA GAA GTA AAC ATA GTG ACA GAC TCA CAA TAT GCA TTG GGA ATC
4020   L   E   V   N   I   V   T   D   S   Q   Y   A   L   G   I   pNL4-3.seq
4020  TTA GAA GTA AAC ATA GTG ACA GAC TCA CAA TAT GCA TTG GGA ATC
4547   L   E   V   N   I   V   T   D   S   Q   Y   A   L   G   I   pHDMHgpm2.seq
4547  CTG GAG GTG AAC ATC GTG ACC GAC TCC CAG TAT GCA TTG GGC ATC

           4070                                              4100
4067   I   Q   A   Q   P   D   K   S   E   S   E   L   V   S   Q   NL4-3 genbank.SEQ
4067  ATT CAA GCA CAA CCA GAT AAG AGT GAA TCA GAG TTA GTC AGT CAA
4065   I   Q   A   Q   P   D   K   S   E   S   E   L   V   S   Q   pNL4-3.seq
4065  ATT CAA GCA CAA CCA GAT AAG AGT GAA TCA GAG TTA GTC AGT CAA
4592   I   Q   A   Q   P   D   K   S   E   S   E   L   V   S   Q   pHDMHgpm2.seq
4592  ATC CAG GCC CAG CCC GAC AAG TCC GAG TCC GAG CTG GTG TCC CAG

                                 4130
4112   I   I   E   Q   L   I   K   K   E   K   V   Y   L   A   W   NL4-3 genbank.SEQ
4112  ATA ATA GAG CAG TTA ATA AAA AAG GAA AAA GTC TAC CTG GCA TGG
4110   I   I   E   Q   L   I   K   K   E   K   V   Y   L   A   W   pNL4-3.seq
4110  ATA ATA GAG CAG TTA ATA AAA AAG GAA AAA GTC TAC CTG GCA TGG
4637   I   I   E   Q   L   I   K   K   E   K   V   Y   L   A   W   pHDMHgpm2.seq
4637  ATC ATC GAG CAG CTG ATC AAG AAG GAG AAG GTG TAC CTG GCC TGG

           4160                                              4190
4157   V   P   A   H   K   G   I   G   G   N   E   Q   V   D   G   NL4-3 genbank.SEQ
4157  GTA CCA GCA CAC AAA GGA ATT GGA GGA AAT GAA CAA GTA GAT GGG
4155   V   P   A   H   K   G   I   G   G   N   E   Q   V   D   K   pNL4-3.seq
4155  GTA CCA GCA CAC AAA GGA ATT GGA GGA AAT GAA CAA GTA GAT AAG
4682   V   P   A   H   K   G   I   G   G   N   E   Q   V   D   K   pHDMHgpm2.seq
4682  GTG CCC GCC CAC AAG GGC ATC GGC GGC AAC GAG CAG GTG GAC AAG

                                 4220
4202   L   V   S   A   G   I   R   K   V   L   F   L   D   G   I   NL4-3 genbank.SEQ
4202  TTG GTC AGT GCT GGA ATC AGG AAA GTA CTA TTT TTA GAT GGA ATA
4200   L   V   S   A   G   I   R   K   V   L   F   L   D   G   I   pNL4-3.seq
4200  TTG GTC AGT GCT GGA ATC AGG AAA GTA CTA TTT TTA GAT GGA ATA
4727   L   V   S   A   G   I   R   K   V   L   F   L   D   G   I   pHDMHgpm2.seq
4727  CTG GTG TCC GCC GGC ATC CGC AAG GTG CTG TTC CTG GAC GGC ATC
```

Fig. 9H

Alignment Report of Codon Optimization (pol).MEG, using Clustal method with PAM250 residue weight table.

4250 4280

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 4247 | D | K | A | Q | E | E | H | E | K | Y | H | S | N | W | R | NL4-3 genbank.SEQ |
| 4247 | GAT | AAG | GCC | CAA | GAA | GAA | CAT | GAG | AAA | TAT | CAC | AGT | AAT | TGG | AGA | |
| 4245 | D | K | A | Q | E | E | H | E | K | Y | H | S | N | W | R | pNL4-3.seq |
| 4245 | GAT | AAG | GCC | CAA | GAA | GAA | CAT | GAG | AAA | TAT | CAC | AGT | AAT | TGG | AGA | |
| 4772 | D | K | A | Q | E | E | H | E | K | Y | H | S | N | W | R | pHDMHgpm2.seq |
| 4772 | GAC | AAG | GCC | CAG | GAG | GAG | CAC | GAG | AAG | TAC | CAC | TCC | AAC | TGG | CGC | |

4310

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 4292 | A | M | A | S | D | F | N | L | P | P | V | V | A | K | E | NL4-3 genbank.SEQ |
| 4292 | GCA | ATG | GCT | AGT | GAT | TTT | AAC | CTA | CCA | CCT | GTA | GTA | GCA | AAA | GAA | |
| 4290 | A | M | A | S | D | F | N | L | P | P | V | V | A | K | E | pNL4-3.seq |
| 4290 | GCA | ATG | GCT | AGT | GAT | TTT | AAC | CTA | CCA | CCT | GTA | GTA | GCA | AAA | GAA | |
| 4817 | A | M | A | S | D | F | N | L | P | P | V | V | A | K | E | pHDMHgpm2.seq |
| 4817 | GCC | ATG | GCC | TCC | GAC | TTC | AAC | CTG | CCC | CCC | GTG | GTG | GCC | AAG | GAG | |

4340 4370

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 4337 | I | V | A | S | C | D | K | C | Q | L | K | G | E | A | M | NL4-3 genbank.SEQ |
| 4337 | ATA | GTA | GCC | AGC | TGT | GAT | AAA | TGT | CAG | CTA | AAA | GGG | GAA | GCC | ATG | |
| 4335 | I | V | A | S | C | D | K | C | Q | L | K | G | E | A | M | pNL4-3.seq |
| 4335 | ATA | GTA | GCC | AGC | TGT | GAT | AAA | TGT | CAG | CTA | AAA | GGG | GAA | GCC | ATG | |
| 4862 | I | V | A | S | C | D | K | C | Q | L | K | G | E | A | M | pHDMHgpm2.seq |
| 4862 | ATC | GTG | GCC | TCC | TGC | GAC | AAG | TGC | CAG | CTG | AAG | GGC | GAG | GCC | ATG | |

4400

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 4382 | H | G | Q | V | D | C | S | P | G | I | W | Q | L | D | C | NL4-3 genbank.SEQ |
| 4382 | CAT | GGA | CAA | GTA | GAC | TGT | AGC | CCA | GGA | ATA | TGG | CAG | CTA | GAT | TGT | |
| 4380 | H | G | Q | V | D | C | S | P | G | I | W | Q | L | D | C | pNL4-3.seq |
| 4380 | CAT | GGA | CAA | GTA | GAC | TGT | AGC | CCA | GGA | ATA | TGG | CAG | CTA | GAT | TGT | |
| 4907 | H | G | Q | V | D | C | S | P | G | I | W | Q | L | D | C | pHDMHgpm2.seq |
| 4907 | CAC | GGC | CAG | GTG | GAC | TGC | TCC | CCC | GGC | ATC | TGG | CAG | CTG | GAC | TGC | |

4430 4460

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 4427 | T | H | L | E | G | K | V | I | L | V | A | V | H | V | A | NL4-3 genbank.SEQ |
| 4427 | ACA | CAT | TTA | GAA | GGA | AAA | GTT | ATC | TTG | GTA | GCA | GTT | CAT | GTA | GCC | |
| 4425 | T | H | L | E | G | K | V | I | L | V | A | V | H | V | A | pNL4-3.seq |
| 4425 | ACA | CAT | TTA | GAA | GGA | AAA | GTT | ATC | TTG | GTA | GCA | GTT | CAT | GTA | GCC | |
| 4952 | T | H | L | E | G | K | V | I | L | V | A | V | H | V | A | pHDMHgpm2.seq |
| 4952 | ACC | CAC | CTG | GAG | GGC | AAG | GTG | ATC | CTG | GTG | GCC | GTG | CAC | GTG | GCC | |

4490

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 4472 | S | G | Y | I | E | A | E | V | I | P | A | E | T | G | Q | NL4-3 genbank.SEQ |
| 4472 | AGT | GGA | TAT | ATA | GAA | GCA | GAA | GTA | ATT | CCA | GCA | GAG | ACA | GGG | CAA | |
| 4470 | S | G | Y | I | E | A | E | V | I | P | A | E | T | G | Q | pNL4-3.seq |
| 4470 | AGT | GGA | TAT | ATA | GAA | GCA | GAA | GTA | ATT | CCA | GCA | GAG | ACA | GGG | CAA | |
| 4997 | S | G | Y | I | E | A | E | V | I | P | A | E | T | G | Q | pHDMHgpm2.seq |
| 4997 | TCC | GGC | TAC | ATC | GAG | GCC | GAG | GTG | ATC | CCC | GCC | GAG | ACC | GGC | CAG | |

Fig. 9I

Alignment Report of Codon Optimization (pol).MEG, using Clustal method with PAM250 residue weight table.

```
          4520                                               4550
4517   E   T   A   Y   F   L   L   K   L   A   G   R   W   P   V    NL4-3 genbank.SEQ
4517  GAA ACA GCA TAC TTC CTC TTA AAA TTA GCA GGA AGA TGG CCA GTA
4515   E   T   A   Y   F   L   L   K   L   A   G   R   W   P   V    pNL4-3.seq
4515  GAA ACA GCA TAC TTC CTC TTA AAA TTA GCA GGA AGA TGG CCA GTA
5042   E   T   A   Y   F   L   L   K   L   A   G   R   W   P   V    pHDMHgpm2.seq
5042  GAG ACC GCC TAC TTC CTG CTG AAG CTG GCC GGC CGC TGG CCC GTG

                                4580
4562   K   T   V   H   T   D   N   G   S   N   F   T   S   T   T    NL4-3 genbank.SEQ
4562  AAA ACA GTA CAT ACA GAC AAT GGC AGC AAT TTC ACC AGT ACT ACA
4560   K   T   V   H   T   D   N   G   S   N   F   T   S   T   T    pNL4-3.seq
4560  AAA ACA GTA CAT ACA GAC AAT GGC AGC AAT TTC ACC AGT ACT ACA
5087   K   T   V   H   T   D   N   G   S   N   F   T   S   T   T    pHDMHgpm2.seq
5087  AAG ACC GTG CAC ACC GAC AAC GGC TCC AAC TTC ACC TCC ACC ACC

          4610                                               4640
4607   V   K   A   A   C   W   W   A   G   I   K   Q   E   F   G    NL4-3 genbank.SEQ
4607  GTT AAG GCC GCC TGT TGG TGG GCG GGG ATC AAG CAG GAA TTT GGC
4605   V   K   A   A   C   W   W   A   G   I   K   Q   E   F   G    pNL4-3.seq
4605  GTT AAG GCC GCC TGT TGG TGG GCG GGG ATC AAG CAG GAA TTT GGC
5132   V   K   A   A   C   W   W   A   G   I   K   Q   E   F   G    pHDMHgpm2.seq
5132  GTG AAG GCC GCC TGC TGG TGG GCC GGC ATC AAG CAG GAG TTC GGC

                                4670
4652   I   P   Y   N   P   Q   S   Q   G   V   I   E   S   M   N    NL4-3 genbank.SEQ
4652  ATT CCC TAC AAT CCC CAA AGT CAA GGA GTA ATA GAA TCT ATG AAT
4650   I   P   Y   N   P   Q   S   Q   G   V   I   E   S   M   N    pNL4-3.seq
4650  ATT CCC TAC AAT CCC CAA AGT CAA GGA GTA ATA GAA TCT ATG AAT
5177   I   P   Y   N   P   Q   S   Q   G   V   I   E   S   M   N    pHDMHgpm2.seq
5177  ATC CCC TAC AAC CCC CAG TCC CAG GGC GTG ATC GAG TCC ATG AAC

          4700                                               4730
4697   K   E   L   K   K   I   I   G   Q   V   R   D   Q   A   E    NL4-3 genbank.SEQ
4697  AAA GAA TTA AAG AAA ATT ATA GGA CAG GTA AGA GAT CAG GCT GAA
4695   K   E   L   K   K   I   I   G   Q   V   R   D   Q   A   E    pNL4-3.seq
4695  AAA GAA TTA AAG AAA ATT ATA GGA CAG GTA AGA GAT CAG GCT GAA
5222   K   E   L   K   K   I   I   G   Q   V   R   D   Q   A   E    pHDMHgpm2.seq
5222  AAG GAG CTG AAG AAG ATC ATC GGC CAA GTC CGC GAC CAG GCC GAG

                                4760
4742   H   L   K   T   A   V   Q   M   A   V   F   I   H   N   F    NL4-3 genbank.SEQ
4742  CAT CTT AAG ACA GCA GTA CAA ATG GCA GTA TTC ATC CAC AAT TTT
4740   H   L   K   T   A   V   Q   M   A   V   F   I   H   N   F    pNL4-3.seq
4740  CAT CTT AAG ACA GCA GTA CAA ATG GCA GTA TTC ATC CAC AAT TTT
5267   H   L   K   T   A   V   Q   M   A   V   F   I   H   N   F    pHDMHgpm2.seq
5267  CAC CTG AAG ACC GCC GTG CAG ATG GCC GTG TTC ATC CAC AAC TTC
```

Fig. 9J

Alignment Report of Codon Optimization (pol).MEG, using Clustal method with PAM250 residue weight table.

```
           4790                                                   4820
4787   K   R   K   G   G   I   G   G   Y   S   A   G   E   R   I   NL4-3 genbank.SEQ
4787  AAA AGA AAA GGG GGG ATT GGG GGG TAC AGT GCA GGG GAA AGA ATA
4785   K   R   K   G   G   I   G   G   Y   S   A   G   E   R   I   pNL4-3.seq
4785  AAA AGA AAA GGG GGG ATT GGG GGG TAC AGT GCA GGG GAA AGA ATA
5312   K   R   K   G   G   I   G   G   Y   S   A   G   E   R   I   pHDMHgpm2.seq
5312  AAG CGC AAG GGC GGC ATC GGC GGC TAC TCC GCC GGC GAG CGC ATC

                                  4850
4832   V   D   I   I   A   T   D   I   Q   T   K   E   L   Q   K   NL4-3 genbank.SEQ
4832  GTA GAC ATA ATA GCA ACA GAC ATA CAA ACT AAA GAA TTA CAA AAA
4830   V   D   I   I   A   T   D   I   Q   T   K   E   L   Q   K   pNL4-3.seq
4830  GTA GAC ATA ATA GCA ACA GAC ATA CAA ACT AAA GAA TTA CAA AAA
5357   V   D   I   I   A   T   D   I   Q   T   K   E   L   Q   K   pHDMHgpm2.seq
5357  GTG GAC ATC ATC GCC ACC GAC ATC CAG ACC AAG GAG CTG CAG AAG

           4880                                                   4910
4877   Q   I   T   K   I   Q   N   F   R   V   Y   Y   R   D   S   NL4-3 genbank.SEQ
4877  CAA ATT ACA AAA ATT CAA AAT TTT CGG GTT TAT TAC AGG GAC AGC
4875   Q   I   T   K   I   Q   N   F   R   V   Y   Y   R   D   S   pNL4-3.seq
4875  CAA ATT ACA AAA ATT CAA AAT TTT CGG GTT TAT TAC AGG GAC AGC
5402   Q   I   T   K   I   Q   N   F   R   V   Y   Y   R   D   S   pHDMHgpm2.seq
5402  CAG ATC ACC AAG ATC CAG AAC TTC CGC GTG TAC TAC CGC GAC TCC

                                  4940
4922   R   D   P   V   W   K   G   P   A   K   L   L   W   K   G   NL4-3 genbank.SEQ
4922  AGA GAT CCA GTT TGG AAA GGA CCA GCA AAG CTC CTC TGG AAA GGT
4920   R   D   P   V   W   K   G   P   A   K   L   L   W   K   G   pNL4-3.seq
4920  AGA GAT CCA GTT TGG AAA GGA CCA GCA AAG CTC CTC TGG AAA GGT
5447   R   D   P   V   W   K   G   P   A   K   L   L   W   K   G   pHDMHgpm2.seq
5447  CGC GAC CCC GTG TGG AAG GGC CCC GCC AAG CTG CTG TGG AAG GGC

           4970                                                   5000
4967   E   G   A   V   V   I   Q   D   N   S   D   I   K   V   V   NL4-3 genbank.SEQ
4967  GAA GGG GCA GTA GTA ATA CAA GAT AAT AGT GAC ATA AAA GTA GTG
4965   E   G   A   V   V   I   Q   D   N   S   D   I   K   V   V   pNL4-3.seq
4965  GAA GGG GCA GTA GTA ATA CAA GAT AAT AGT GAC ATA AAA GTA GTG
5492   E   G   A   V   V   I   Q   D   N   S   D   I   K   V   V   pHDMHgpm2.seq
5492  GAG GGC GCC GTG GTG ATC CAG GAC AAC TCC GAC ATC AAG GTG GTG

                                  5030
5012   P   R   R   K   A   K   I   I   R   D   Y   G   K   Q   M   NL4-3 genbank.SEQ
5012  CCA AGA AGA AAA GCA AAG ATC ATC AGG GAT TAT GGA AAA CAG ATG
5010   P   R   R   K   A   K   I   I   R   D   Y   G   K   Q   M   pNL4-3.seq
5010  CCA AGA AGA AAA GCA AAG ATC ATC AGG GAT TAT GGA AAA CAG ATG
5537   P   R   R   K   A   K   I   I   R   D   Y   G   K   Q   M   pHDMHgpm2.seq
5537  CCC CGC CGC AAG GCC AAG ATC ATC CGC GAC TAC GGC AAG CAG ATG
```

Fig. 9K

Alignment Report of Codon Optimization (pol).MEG, using Clustal method with PAM250 residue weight table.

| | 5060 | | | | | | | | | 5090 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 5057 | A | G | D | D | C | V | A | S | R | Q | D | E | D | | NL4-3 genbank.SEQ |
| 5057 | GCA | GGT | GAT | GAT | TGT | GTG | GCA | AGT | AGA | CAG | GAT | GAG | GAT | TAA | |
| 5055 | A | G | D | D | C | V | A | S | R | Q | D | E | D | | pNL4-3.seq |
| 5055 | GCA | GGT | GAT | GAT | TGT | GTG | GCA | AGT | AGA | CAG | GAT | GAG | GAT | TAA | |
| 5582 | A | G | D | D | C | V | A | S | R | Q | D | E | D | | pHDMHgpm2.seq |
| 5582 | GCC | GGC | GAC | GAC | TGC | GTG | GCC | TCC | CGC | CAG | GAC | GAG | GAC | TAA | |

Fig. 9L

AGCTTGGCCC ATTGCATACG TTGTATCCAT ATCATAATAT GTACATTTAT ATTGGCTCAT 60
GTCCAACATT ACCGCCATGT TGACATTGAT TATTGACTAG TTATTAATAG TAATCAATTA 120
CGGGGTCATT AGTTCATAGC CCATATATGG AGTTCCGCGT TACATAACTT ACGGTAAATG 180
GCCCGCCTGG CTGACCGCCC AACGACCCCC GCCCATTGAC GTCAATAATG ACGTATGTTC 240
CCATAGTAAC GCCAATAGGG ACTTTCCATT GACGTCAATG GGTGGAGTAT TTACGGTAAA 300
CTGCCCACTT GGCAGTACAT CAAGTGTATC ATATGCCAAG TACGCCCCCT ATTGACGTCA 360
ATGACGGTAA ATGGCCCGCC TGGCATTATG CCCAGTACAT GACCTTATGG GACTTTCCTA 420
CTTGGCAGTA CATCTACGTA TTAGTCATCG CTATTACCAT GGTGATGCGG TTTTGGCAGT 480
ACATCAATGG GCGTGGATAG CGGTTTGACT CACGGGGATT TCCAAGTCTC CACCCCATTG 540
ACGTCAATGG GAGTTTGTTT TGGCACCAAA ATCAACGGGA CTTTCCAAAA TGTCGTAACA 600
ACTCCGCCCC ATTGACGCAA ATGGGCGGTA GGCGTGTACG GTGGGAGGTC TATATAAGCA 660
GAGCTCGTTT AGTGAACCGT CAGATCGCCT GGAGACGCCA TCCACGCTGT TTTGACCTCC 720
ATAGAAGACA CCGGGACCGA TCCAGCCTCC CCTCGAAGCT GATCCTGAGA ACTTCAGGGT 780
GAGTCTATGG GACCCTTGAT GTTTTCTTTC CCCTTCTTTT CTATGGTTAA GTTCATGTCA 840
TAGGAAGGGG AGAAGTAACA GGGTACACAT ATTGACCAAA TCAGGGTAAT TTTGCATTTG 900
TAATTTTAAA AAATGCTTTC TTCTTTTAAT ATACTTTTTT GTTTATCTTA TTTCTAATAC 960
TTTCCCTAAT CTCTTTCTTT CAGGGCAATA ATGATACAAT GTATCATGCC TCTTTGCACC 1020
ATTCTAAAGA ATAACAGTGA TAATTTCTGG GTTAAGGCAA TAGCAATATT TCTGCATATA 1080
AATATTTCTG CATATAAATT GTAACTGATG TAAGAGGTTT CATATTGCTA ATAGCAGCTA 1140
CAATCCAGCT ACCATTCTGC TTTTATTTTA TGGTTGGGAT AAGGCTGGAT TATTCTGAGT 1200
CCAAGCTAGG CCCTTTTGCT AATCATGTTC ATACCTCTTA TCTTCCTCCC ACAGCTCCTG 1260
GGCAACGTGC TGGTCTGTGT GCTGGCCCAT CACTTTGGCA AAGAATTCTA GACTGCCATG 1320
GGCGCCCGCG CCTCCGTGCT GTCCGGCGGC GAGCTGGACA AGTGGGAGAA GATCCGCCTG 1380
CGCCCCGGCG GCAAGAAGCA GTACAAGCTG AAGCACATCG TGTGGGCCTC CCGCGAGCTG 1440
GAGCGCTTCG CCGTGAACCC CGGCCTGCTG GAGACCTCCG AGGGCTGCCG CCAGATCCTG 1500
GGCCAGCTGC AGCCCTCCCT GCAAACCGGC TCCGAGGAGC TGCGCTCCCT GTACAACACC 1560
ATCGCCGTGC TGTACTGCGT GCACCAGCGC ATCGACGTGA AGGACACCAA GGAGGCCCTG 1620
GACAAGATCG AGGAGGAGCA GAACAAGTCC AAGAAGAAGG CCCAGCAGGC CGCCGCCGAC 1680
ACCGGCAACA ACTCCCAGGT GTCCCAGAAC TACCCCATCG TGCAGAACCT GCAGGGCCAG 1740
ATGGTGCACC AGGCCATCTC CCCCCGCACC CTGAACGCCT GGGTGAAGGT GGTGGAGGAG 1800
AAGGCCTTCT CCCCCGAAGT CATCCCCATG TTCTCCGCCC TGTCCGAGGG CGCCACCCCC 1860
CAGGACCTGA ACACCATGCT GAACACCGTG GGCGGCCACC AGGCCGCCAT GCAGATGCTG 1920
AAGGAGACCA TCAACGAGGA GGCCGCCGAG TGGGACCGCC TGCACCCCGT GCACGCCGGC 1980
CCCATCGCCC CCGGCCAGAT GCGCGAGCCC CGCGGCTCCG ACATCGCCGG CACCACCTCC 2040
ACCCTGCAAG AGCAGATCGG CTGGATGACC CACAACCCCC CCATCCCCGT GGGCGAGATC 2100
TACAAGCGCT GGATCATCCT GGGCCTGAAC AAGATCGTGC GCATGTACTC CCCCACCTCC 2160
ATCCTGGACA TCCGCCAGGG CCCCAAGGAG CCCTTCCGCG ACTACGTGGA CCGCTTCTAC 2220
AAGACCCTGC GCGCCGAGCA GGCCTCCCAG GAGGTAAAGA ACTGGATGAC CGAGACCCTG 2280
CTGGTGCAGA ACGCCAACCC CGACTGCAAG ACCATCCTGA AGGCCCTGGG CCCCGGCGCC 2340
ACCCTGGAGG AGATGATGAC CGCCTGCCAG GGCGTGGGCG GCCCCGGCCA CAAGGCCCGC 2400
GTGCTGGCCG AGGCCATGTC CCAAGTCACC AACCCCGCCA CCATCATGAT CCAGAAGGGC 2460
AACTTCCGCA ACCAGCGCAA GACCGTGAAG TGCTTCAACT GCGGCAAGGA GGGCCACATC 2520
GCCAAGAACT GCCGCGCCCC CCGCAAGAAG GGCTGCTGGA AGTGCGGCAA GGAGGGCCAC 2580
CAGATGAAAG ATTGTACTGA GAGACAGGCT AATTTTTTAG GGAAGATCTG GCCTTCCCAC 2640
AAGGGAAGGC CAGGGAATTT TCTTCAGAGC AGACCAGAGC CAACAGCCCC ACCAGAAGAG 2700
AGCTTCAGGT TTGGGGAAGA GACAACAACT CCCTCTCAGA AGCAGGAGCC GATAGACAAG 2760
GAACTGTATC CTTTAGCTTC CCTCAGATCA CTCTTTGGCA GCGACCCCTC GTCACAATAA 2820

Fig. 10A

```
AGATCGGTGG CCAGCTGAAG GAGGCCCTGC TGGACACCGG CGCCGACGAC ACCGTGCTGG 2880
AGGAGATGAA CCTGCCCGGC CGCTGGAAGC CCAAGATGAT CGGCGGCATC GGCGGCTTCA 2940
TCAAAGTCCG CCAGTACGAC CAGATCCTGA TCGAGATCTG CGGCCACAAG GCCATCGGCA 3000
CCGTGCTGGT GGGCCCCACC CCCGTGAACA TCATCGGCCG CAACCTGCTG ACCCAGATCG 3060
GCTGCACCCT GAACTTCCCC ATCTCCCCCA TCGAGACCGT GCCCGTGAAG CTGAAGCCCG 3120
GCATGGACGG CCCCAAAGTC AAGCAGTGGC CCCTGACCGA GGAGAAGATC AAGGCCCTGG 3180
TGGAGATCTG CACCGAGATG GAGAAGGAGG GCAAGATCTC CAAGATCGGC CCCGAGAACC 3240
CCTACAACAC CCCCGTGTTC GCCATCAAGA AGAAGGACTC CACCAAGTGG CGCAAGCTGG 3300
TGGACTTCCG CGAGCTGAAC AAGCGCACCC AGGACTTCTG GGAGGTGCAG CTGGGCATCC 3360
CCCACCCCGC CGGCCTGAAG CAGAAGAAGT CCGTGACCGT GCTGGACGTG GGCGACGCCT 3420
ACTTCTCCGT GCCCCTGGAC AAGGACTTCC GCAAGTACAC CGCCTTCACC ATCCCCTCCA 3480
TCAACAACGA GACCCCCGGC ATCCGCTACC AGTACAACGT GCTGCCCCAG GGCTGGAAGG 3540
GCTCCCCCGC CATCTTCCAG TGCTCCATGA CCAAGATCCT GGAGCCCTTC CGCAAGCAGA 3600
ACCCCGACAT CGTGATCTAC CAGTACATGG ACGACCTGTA CGTGGGCTCC GACCTGGAGA 3660
TCGGCCAGCA CCGCACCAAG ATCGAGGAGC TGCGCCAGCA CCTGCTGCGC TGGGGCTTCA 3720
CCACCCCCGA CAAGAAGCAC CAGAAGGAGC CCCCCTTCCT GTGGATGGGC TACGAGCTGC 3780
ACCCCGACAA GTGGACCGTG CAGCCCATCG TGCTGCCCGA GAAGGACTCC TGGACCGTGA 3840
ACGACATCCA GAAGCTGGTG GGCAAGCTGA ACTGGGCCTC CCAGATCTAC GCCGGCATCA 3900
AAGTCCGCCA GCTGTGCAAG CTGCTGCGCG GCACCAAGGC CCTGACCGAG GTGGTGCCCC 3960
TGACCGAGGA GGCCGAGCTG GAGCTGGCCG AGAACCGCGA GATCCTGAAG GAGCCCGTGC 4020
ACGGCGTGTA CTACGACCCC TCCAAGGACC TGATCGCCGA GATCCAGAAG CAGGGCCAGG 4080
GCCAGTGGAC CTACCAGATC TACCAGGAGC CCTTCAAGAA CCTGAAGACC GGCAAATACG 4140
CCCGCATGAA GGGCGCCCAC ACCAACGACG TGAAGCAGCT GACCGAGGCC GTGCAGAAGA 4200
TCGCCACCGA GTCCATCGTG ATCTGGGGCA AGACTCCCAA GTTCAAGCTG CCCATCCAGA 4260
AGGAGACCTG GGAGGCCTGG TGGACCGAGT ACTGGCAGGC CACCTGGATC CCCGAGTGGG 4320
AGTTCGTGAA CACCCCCCCC CTGGTGAAGC TGTGGTACCA GCTGGAGAAG GAGCCCATCA 4380
TCGGCGCCGA GACCTTCTAC GTGGACGGCG CCGCCAACCG CGAGACCAAG CTGGGCAAGG 4440
CCGGCTACGT GACCGACCGC GGCCGCCAGA AGGTGGTGCC CCTGACCGAC ACCACCAACC 4500
AGAAGACCGA GCTGCAGGCC ATCCACCTGG CCCTGCAAGA CTCCGGCCTG GAGGTGAACA 4560
TCGTGACCGA CTCCCAGTAT GCATTGGGCA TCATCCAGGC CCAGCCCGAC AAGTCCGAGT 4620
CCGAGCTGGT GTCCCAGATC ATCGAGCAGC TGATCAAGAA GGAGAAGGTG TACCTGGCCT 4680
GGGTGCCCGC CCACAAGGGC ATCGGCGGCA ACGAGCAGGT GGACAAGCTG GTGTCCGCCG 4740
GCATCCGCAA GGTGCTGTTC CTGGACGGCA TCGACAAGGC CCAGGAGGAG CACGAGAAGT 4800
ACCACTCCAA CTGGCGCGCC ATGGCCTCCG ACTTCAACCT GCCCCCCGTG GTGGCCAAGG 4860
AGATCGTGGC CTCCTGCGAC AAGTGCCAGC TGAAGGGCGA GGCCATGCAC GGCCAGGTGG 4920
ACTGCTCCCC CGGCATCTGG CAGCTGGACT GCACCCACCT GGAGGGCAAG GTGATCCTGG 4980
TGGCCGTGCA CGTGGCCTCC GGCTACATCG AGGCCGAGGT GATCCCCGCC GAGACCGGCC 5040
AGGAGACCGC CTACTTCCTG CTGAAGCTGG CCGGCCGCTG GCCCGTGAAG ACCGTGCACA 5100
CCGACAACGG CTCCAACTTC ACCTCCACCA CCGTGAAGGC CGCCTGCTGG TGGGCCGGCA 5160
TCAAGCAGGA GTTCGGCATC CCCTACAACC CCCAGTCCCA GGGCGTGATC GAGTCCATGA 5220
ACAAGGAGCT GAAGAAGATC ATCGGCCAAG TCCGCGACCA GGCCGAGCAC CTGAAGACCG 5280
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CCGACATCAA GGTGGTGCCC CGCCGCAAGG CCAAGATCAT CCGCGACTAC GGCAAGCAGA 5580
TGGCCGGCGA CGACTGCGTG GCCTCCCGCC AGGACGAGGA CTAACACATG GAAAAGATTA 5640
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Fig. 10B

GTAAAACACC ATAGGCCGCT CTAGAGGATC CAAGCTTATC GATACCGTCG ACCTCGAGGG 5700
CCCAGATCTA ATTCACCCCA CCAGTGCAGG CTGCCTATCA GAAAGTGGTG GCTGGTGTGG 5760
CTAATGCCCT GGCCCACAAG TATCACTAAG CTCGCTTTCT TGCTGTCCAA TTTCTATTAA 5820
AGGTTCCTTT GTTCCCTAAG TCCAACTACT AAACTGGGGG ATATTATGAA GGGCCTTGAG 5880
CATCTGGATT CTGCCTAATA AAAAACATTT ATTTTCATTG CAATGATGTA TTTAAATTAT 5940
TTCTGAATAT TTTACTAAAA AGGGAATGTG GGAGGTCAGT GCATTTAAAA CATAAAGAAA 6000
TGAAGAGCTA GTTCAAACCT TGGGAAAATA CACTATATCT TAAACTCCAT GAAAGAAGGT 6060
GAGGCTGCAA ACAGCTAATG CACATTGGCA ACAGCCCCTG ATGCCTATGC CTTATTCATC 6120
CCTCAGAAAA GGATTCAAGT AGAGGCTTGA TTTGGAGGTT AAAGTTTTGC TATGCTGTAT 6180
TTTACATTAC TTATTGTTTT AGCTGTCCTC ATGAATGTCT TTTCACTACC CATTTGCTTA 6240
TCCTGCATCT CTCAGCCTTG ACTCCACTCA GTTCTCTTGC TTAGAGATAC CACCTTTCCC 6300
CTGAAGTGTT CCTTCCATGT TTTACGGCGA GATGGTTTCT CCTCGCCTGG CCACTCAGCC 6360
TTAGTTGTCT CTGTTGTCTT ATAGAGGTCT ACTTGAAGAA GGAAAAACAG GGGGCATGGT 6420
TTGACTGTCC TGTGAGCCCT TCTTCCCTGC CTCCCCCACT CACAGTGACC CGGAATCCCT 6480
CGACATGGCA GTCTAGATCA TTCTTGAAGA CGAAAGGGCC TCGTGATACG CCTATTTTTA 6540
TAGGTTAATG TCATGATAAT AATGGTTTCT TAGACGTCAG GTGGCACTTT TCGGGGAAAT 6600
GTGCGCGGAA CCCCTATTTG TTTATTTTTC TAAATACATT CAAATATGTA TCCGCTCATG 6660
AGACAATAAC CCTGATAAAT GCTTCAATAA TATTGAAAAA GGAAGAGTAT GAGTATTCAA 6720
CATTTCCGTG TCGCCCTTAT TCCCTTTTTT GCGGCATTTT GCCTTCCTGT TTTTGCTCAC 6780
CCAGAAACGC TGGTGAAAGT AAAAGATGCT GAAGATCAGT TGGGTGCACG AGTGGGTTAC 6840
ATCGAACTGG ATCTCAACAG CGGTAAGATC CTTGAGAGTT TTCGCCCCGA AGAACGTTTT 6900
CCAATGATGA GCACTTTTAA AGTTCTGCTA TGTGGCGCGG TATTATCCCG TATTGACGCC 6960
GGGCAAGAGC AACTCGGTCG CCGCATACAC TATTCTCAGA ATGACTTGGT TGAGTACTCA 7020
CCAGTCACAG AAAAGCATCT TACGGATGGC ATGACAGTAA GAGAATTATG CAGTGCTGCC 7080
ATAACCATGA GTGATAACAC TGCGGCCAAC TTACTTCTGA CAACGATCGG AGGACCGAAG 7140
GAGCTAACCG CTTTTTTGCA CAACATGGGG GATCATGTAA CTCGCCTTGA TCGTTGGGAA 7200
CCGGAGCTGA ATGAAGCCAT ACCAAACGAC GAGCGTGACA CCACGATGCC TGTAGCAATG 7260
GCAACAACGT TGCGCAAACT ATTAACTGGC GAACTACTTA CTCTAGCTTC CCGGCAACAA 7320
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GCTGGCTGGT TTATTGCTGA TAAATCTGGA GCCGGTGAGC GTGGGTCTCG CGGTATCATT 7440
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CATTGGTAAC TGTCAGACCA AGTTTACTCA TATATACTTT AGATTGATTT AAAACTTCAT 7620
TTTTAATTTA AAAGGATCTA GGTGAAGATC CTTTTTGATA ATCTCATGAC CAAAATCCCT 7680
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TGAGATCCTT TTTTTCTGCG CGTAATCTGC TGCTTGCAAA CAAAAAAACC ACCGCTACCA 7800
GCGGTGGTTT GTTTGCCGGA TCAAGAGCTA CCAACTCTTT TTCCGAAGGT AACTGGCTTC 7860
AGCAGAGCGC AGATACCAAA TACTGTTCTT CTAGTGTAGC CGTAGTTAGG CCACCACTTC 7920
AAGAACTCTG TAGCACCGCC TACATACCTC GCTCTGCTAA TCCTGTTACC AGTGGCTGCT 7980
GCCAGTGGCG ATAAGTCGTG TCTTACCGGG TTGGACTCAA GACGATAGTT ACCGGATAAG 8040
GCGCAGCGGT CGGGCTGAAC GGGGGGTTCG TGCACACAGC CCAGCTTGGA GCGAACGACC 8100
TACACCGAAC TGAGATACCT ACAGCGTGAG CTATGAGAAA GCGCCACGCT TCCCGAAGGG 8160
AGAAAGGCGG ACAGGTATCC GGTAAGCGGC AGGGTCGGAA CAGGAGAGCG CACGAGGGAG 8220
CTTCCAGGGG GAAACGCCTG GTATCTTTAT AGTCCTGTCG GGTTTCGCCA CCTCTGACTT 8280
GAGCGTCGAT TTTTGTGATG CTCGTCAGGG GGGCGGAGCC TATGGAAAAA CGCCAGCAAC 8340
GGATGCGCCG CGTGCGGCTG CTGGAGATGG CGGACGCGAT GGATATGTTC TGCCAAGGGT 8400
TGGTTTGCGC ATTCACAGTT CTCCGCAAGA ATTGATTGGC TCCAATTCTT GGAGTGGTGA 8460

Fig. 10C

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ATCCGTTAGC GAGGTGCCGC CGGCTTCCAT TCAGGTCGAG GTGGCCCGGC TCCATGCACC  8520
GCGACGCAAC GCGGGGAGGC AGACAAGGTA TAGGGCGGCG CCTACAATCC ATGCCAACCC  8580
GTTCCATGTG CTCGCCGAGG CGGCATAAAT CCCCGTGACG ATCAGCGGTC CAATGATCGA  8640
AGTTAGGCTG GTAAGAGCCG CGAGCGATCC TTGAAGCTGT CCCTGATGGT CGTCATCTAC  8700
CTGCCTGGAC AGCATGGCCT GCAACGCGGG CATCCCGATG CCGCCGGAAG CGAGAAGAAT  8760
CATAATGGGG AAGGCCATCC AGCCTCGCGT CGGGGAGCTT TTTGCAAAAG CCTAGGCCTC  8820
CAAAAAAGCC TCCTCACTAC TTCTGGAATA GCTCAGAGGC CGAGGCGGCC TCGGCCTCTG  8880
CATAAATAAA AAAAATTAGT CAGCCATG  8908
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Fig. 10D

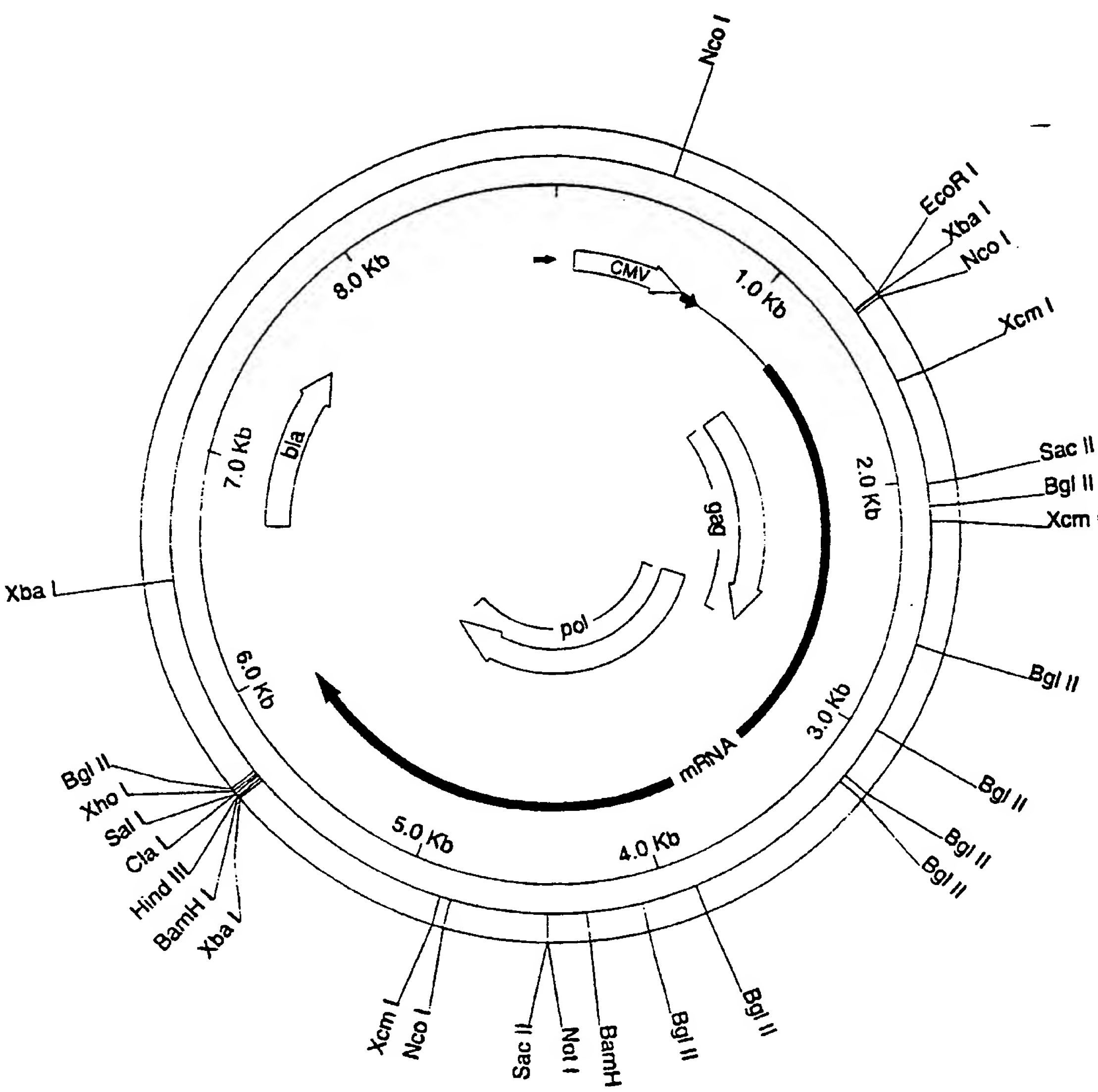
Nco I
EcoR I
Xba I
Nco I
Xcm I
Sac II
Bgl II
Xcm I
Bgl II
Bgl II
Bgl II
Bgl II
Bgl II
Bgl II
BamH I
Not I
Sac II
Nco I
Xcm I
Xba I
BamH I
Hind III
Cla I
Sal I
Xho I
Bgl II
Xba I
1.0 Kb
2.0 Kb
3.0 Kb
4.0 Kb
5.0 Kb
6.0 Kb
7.0 Kb
8.0 Kb
CMV
gag
pol
mRNA
bla

Fig. 11

# INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/20675

**A. CLASSIFICATION OF SUBJECT MATTER**
IPC 7 C12N15/86 C12N5/10 C12N7/04 C12N15/49 C07K14/16

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | NALDINI L ET AL: "IN VIVO GENE DELIVERY AND STABLE TRANSDUCTION OF NONDIVIDING CELLS BY A LENTIVIRAL VECTOR" SCIENCE,US,AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE,, vol. 272, no. 5259, 12 April 1996 (1996-04-12), pages 263-267, XP000583652 ISSN: 0036-8075 cited in the application the whole document --- | 1-4 |

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[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 25 February 2000 | 03/03/2000 |

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| European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016 | Chambonnet, F |

1

# INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/20675

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication,where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | HASELHORST D ET AL: "STABLE PACKAGING CELL LINES AND HIV-1 BASED RETROVIRAL VECTOR SYSTEMS"<br>GENE THERAPY,GB,MACMILLAN PRESS LTD., BASINGSTOKE,<br>vol. 1, no. SUPPL. 02,<br>18 November 1994 (1994-11-18), page S14<br>XP002063698<br>ISSN: 0969-7128<br>the whole document<br>--- | |
| A | ST LOUIS D ET AL: "CONSTRUCTION AND CHARACTERIZATION OF HIV-1 RETROVIRAL VECTORS AND REPLICATION-DEFECTIVE HIV-1 PACKAGING CELL LINES"<br>INTERNATIONAL CONFERENCE ON AIDS AND THE STD WORLD CONGRESS,XX,XX,<br>1 June 1993 (1993-06-01), page 244<br>XP002063695<br>the whole document<br>--- | 1 |
| A | CARROLL R ET AL: "A HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 (HIV-1)-BASED RETROVIRAL VECTOR SYSTEM UTILIZING STABLE HIV-1 PACKAGING CELL LINES"<br>JOURNAL OF VIROLOGY,US,THE AMERICAN SOCIETY FOR MICROBIOLOGY,<br>vol. 68, no. 9,<br>1 September 1994 (1994-09-01), pages 6047-6051, XP002063697<br>ISSN: 0022-538X<br>the whole document<br>--- | 1 |
| X | HOLLER T P ET AL: "HIV1 INTEGRASE EXPRESSED IN ESCHERICHIA COLI FROM A SYNTHETIC GENE"<br>GENE,NL,ELSEVIER BIOMEDICAL PRESS. AMSTERDAM,<br>vol. 136, 22 December 1993 (1993-12-22), pages 323-328, XP000199775<br>ISSN: 0378-1119<br>the whole document<br>--- | 39 |
| A | ANDRE S ET AL: "INCREASED IMMUNE RESPONSE ELICITED BY DNA VACCINATION WITH A SYNTHETIC GP120 SEQUENCE WITH OPTIMIZED CODON USAGE"<br>JOURNAL OF VIROLOGY,US,THE AMERICAN SOCIETY FOR MICROBIOLOGY,<br>vol. 72, no. 2,<br>1 February 1998 (1998-02-01), pages 1497-1503, XP002073767<br>ISSN: 0022-538X<br>the whole document<br>----- | 39-43 |